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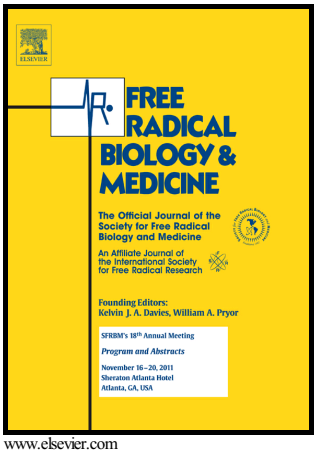

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Dynamic Regulation of Epigenetic Demethylation by Oxygen Availability and Cellular Redox

Nermina Lamadema, Simon Burr and Alison C. Brewer

School of Cardiovascular Medicine & Sciences

King's College London BHF Centre of Research Excellence

Corresponding Author:

Dr Alison Brewer

Department of Cardiovascular Sciences

The James Black Centre

125 Coldharbour Lane

London SE5 9NU

Email: alison.brewer@kcl.ac.uk

Abstract

The chromatin structure of the mammalian genome must facilitate both precisely-controlled DNA replication together with tightly-regulated gene transcription. This necessarily involves complex mechanisms and processes which remain poorly understood. It has long been recognised that the epigenetic landscape becomes established during embryonic development and acts to specify and determine cell fate. In addition, the chromatin structure is highly dynamic and allows for both cellular reprogramming and homeostatic modulation of cell function. In this respect, the functions of epigenetic "erasers", which act to remove covalently-linked epigenetic modifications from DNA and histones are critical. The enzymatic activities of the TET and JmjC protein families have been identified as demethylases which act to remove methyl groups from DNA and histones, respectively. Further, they are characterised as members of the Fe(II)- and 2-oxoglutarate-dependent dioxygenase superfamily. This provides the intriguing possibility that their enzymatic activities may be modulated by cellular metabolism, oxygen availability and redox-based mechanisms, all of which are likely to display dynamic cell- and tissue-specific patterns of flux. Here we discuss the current evidence for such [O_2]- and redox-dependent regulation of the TET and Jmjc demethylases and the potential physiological and pathophysiological functional consequences of such regulation.

Keywords:

Epigenetics; Demethylation; Hypoxia; Cellular Redox; Tet proteins; JmjC proteins.

Introduction

The DNA comprising the genome of a single human (diploid) cell comprises more than 6 billion base pairs which, if stretched end to end, would extend to about 2 metres. That this becomes packaged into a nucleus of approximately 6 μm in diameter, in such a way that allows its code to be used to control every function of the cell, seems truly remarkable. Eukaryotic genomic DNA is hierarchically compacted into chromatin. It is wound around octamers of histone proteins (consisting of 2 copies of each of the core histones: H2A,H2B H3 and H4) to form a linear nucleosomal array which is then further folded and organised by association with linker histones to generate higher-order chromatin structures [1]. Eukaryotic DNA is compartmentalised into the histologically-distinct forms of highly-condensed (inaccessible, "repressive") heterochromatin and the more loosely-packed (accessible, "active") euchromatin [2]. As many as five structural sub-types of chromatin have been identified in *Drosophila* cells (two types of "active" chromatin, and 3 types of "repressive" chromatin) [3]. It is also clear that both heterochromatin and euchromatin can be further subdivided and characterised in mammalian cells [4]. It is now recognised that the structure of chromatin is highly dynamic [5] and that this plasticity in the degree of chromatin condensation is essential to facilitate the regulation of cellular processes which necessitate access of catalytic proteins to DNA, such as DNA replication, transcription and DNA repair.

The term "epigenetic" roughly summarizes all changes at the nuclear and mitochondrial DNA (nDNA and mtDNA) or RNA level, which alter their structure or conformation, but not their primary sequence, and affect gene expression and hence cellular function[6]. Thus, to a large extent, the study of epigenetics involves understanding the mechanisms involved in regulating the remodelling of chromatin structure to facilitate accessibility of specific gene loci to their cognate transcription factors. Epigenetic regulatory mechanisms are therefore fundamental to the acquisition of cell fate during embryonic development and the mediation of physiological cellular homeostasis. Further, the dysregulation of epigenetic processes correlate with, and may be causal in, the development and progression of disease, and most notably cancer [7].

Epigenetic Modifications affecting Chromatin Structure

The degree of compaction and hence accessibility of specific genetic loci can be regulated by both the replacement of the canonical core histones (H2A, H2B H3 and H4) with histone variants (reviewed in [8]) and ATP-dependent chromatin remodelling complexes (reviewed in [9]). In addition, reversible covalent modifications of the genomic DNA itself and of the N-terminal tails of the histone proteins are critical processes involved in the regulation of chromatin structure. In the case of the DNA, the most prevalent covalent modification is methylation of the C5-position of cytosine (5-methylcytosine; 5mC), predominantly at CpG dinucleotides [10]. Within the human genome, about 70% of CpG sites are methylated, but this equates to only about 5% of all cytosine residues [11]. Thus, CpG dinucleotides occur relatively infrequently and are typically concentrated in short CpG-rich DNA tracts termed "CpG islands". Most human gene promoters (50–70%) are embedded within such CpG islands and their methylation is normally associated with transcriptional repression [10]. Consequently, analyses of differentially-methylated DNA loci have shown the co-localisation of hypomethylated regions with promoters and enhancers of actively transcribed, tissue-specific and developmental stage-specific genes [12, 13].

By contrast to DNA epigenetic modification, there is an enormous complexity of possible post-translational modifications of histone proteins. In addition to methylation, different histone proteins can be subject to acetylation, phosphorylation, ubiquitination, GlcNAcylation, citrullination, hydroxylation and SUMOylation [14], which each elicit specific (both positive and negative) effects upon chromatin compaction and structure and hence gene function. There is also far greater complexity with the regard to both the location and degree of each specific modification. All histones can theoretically be methylated at arginine and lysine residues, and an ever-increasing number of residues have been empirically shown to be targeted [15-19]. However, some residues are more frequently methylated, such as Lysine (K)-4, -9, -27, -36 and -79 of histone H3, and K-20 of histone H4 (reviewed in [20]). In mammalian cells, di-methylation is most common (an estimated 40-80% of all histones are di-methylated at H3K9, H3K27, H3K36 and H4K20), while mono- and tri-methylation modifications of these residues are less abundant (affecting approximately 20% of histones) [21, 22]. The structures and functions of the epigenetic modifiers which act to add or remove these modifications are becoming increasingly well characterised [23]. However, the mechanisms that underlie the enormous complexity of their dynamic regulation of function in (patho)-physiological cellular processes are not understood.

DNA and Histone Methylation; Epigenetic Regulation by Members of the 2-oxoglutarate-dependent Dioxygenase Superfamily

The chromosomal patterns of both DNA and histone methylation are maintained by the opposing actions of enzymatic methylase and demethylase systems, termed epigenetic "writers" and "erasers" respectively [24]. In the case of DNA, methylation at CpG dinucleotides is achieved by DNA methyltransferases (DNMTs) of which there are 3 in mammals, with proven and essential methyltransferase activity; DNMT1, DNMT3a and DNMT3b [25]. These catalyse the transfer of a methyl group from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the C5 position of cytosine. DNMT1 acts solely upon hemimethylated DNA and therefore acts to maintain methylation status after DNA replication, while DNMT3a and DNMT3b are additionally involved in *de novo* DNA methylation [25]. Similarly, SAM is utilised in the methylation of histone proteins by three distinct histone methyltransferase (HMT) enzymatic systems: SET domain lysine methyltransferases, non-SET domain lysine methyltransferases and arginine methyltransferases (reviewed in [26]). Although methylation of the histone tails does not alter the charge of the proteins, it increases their hydrophobicity and hence can alter the affinity of specific proteins such as transcription factors for their cognate DNA binding sites [27].

It is, perhaps, noteworthy that the activities of both the DNMTs and the HMTs are dependent upon the intracellular levels of SAM, which fluctuate based upon the cellular nutrient availability [28]. This therefore potentially provides a sensory-mechanistic link between metabolism, energy homeostasis and the regulation of gene expression by methylation of histones and DNA.

For many years, the methylation of both DNA and histone proteins were thought to be irreversible modifications, which would be lost from the chromatin only by "passive" mechanisms during DNA replication. However, more recently, active demethylation enzymatic processes have been characterised, which are predominantly catalysed by members of the superfamily of Fe(II)-, and 2-oxoglutarate-dependent dioxygenases (2-OGDDs) [29]. 2-OGDDs are widely distributed throughout the animal and plant kingdoms, and more than 60 such enzymes have been identified in humans alone [30]. These are involved in diverse biochemical roles associated with fatty acid metabolism, carnitine biosynthesis and phytanic acid catabolism [31]. They catalyse a variety of oxidative transformations, of which the most studied and well-established (and that which is relevant here) is hydroxylation of a respective substrate (reviewed in [32]). The absolute requirement of these

enzymes for 2-oxoglutarate (2-OG) may (as in the case of the methylases) provide a link between the cellular metabolic status and their enzymatic activity. In addition, their requirement for molecular oxygen might suggest potential roles as O_2 sensors, while the necessity for Fe(II), (and inhibition by Fe(III)), may additionally suggest potential regulation by cellular-redox status and/or redox-signalling mechanisms. These intriguing characteristics may therefore facilitate roles of these demethylases in the epigenetic-determination of specific patterns of gene expression, both during normal cellular development and in the physiological homeostatic responses to cellular stresses. In addition, they may provide mechanistic insights into the epigenetic dysregulation observed during disease.

DNA Demethylation by Ten-Eleven Translocation Enzymes (TETs)

The 5mC modification was found not to be a stable, irreversible modification of DNA, when the significant and unexpected observation was made that the ten eleven translocation protein 1 (TET1) was able to catalyse its conversion to 5-hydroxymethyl cytosine (5hmC) [33]. TET1 had originally been cloned and characterised as an acute myeloid leukaemia-associated protein of unknown physiological function, which translocated to generate a fusion partner with mixed-lineage leukaemia (MLL) [34, 35]. Its translocation from chromosome ten to chromosome eleven determined its nomenclature. Based on sequence homology, two additional paralogues were identified in human and mouse (TET2 and TET3) [34]. Sequence homology between TET proteins and the trypanosome proteins, JBP1 and JBP2, which are 2-OGDD enzymes that were believed to oxidise the 5-methyl group of thymine, led to the proposal of the involvement of TET proteins in epigenetic modification [33]. Subsequently, the occurrence of TET-dependent 5hmC within the mammalian genome confirmed this. Thus, it is now recognised that TET1, TET2 and TET3 all catalyse successive oxidations of 5mC to 5hmC, 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [36, 37]. These oxidised cytosine modifications can subsequently be lost upon DNA replication. Alternatively, active demethylation can be achieved by thymine DNA glycosylase (TDG)-dependent removal of 5fC and 5caC, coupled with base excision repair (reviewed in[38], see Figure 1A).

In addition to their presence as intermediates in the demethylation process, these oxidised forms are also recognised as relatively stable epigenetic modifications in the genomic DNA of both dividing and non-dividing cells [39, 40], with specific regulatory functions that are increasingly being elucidated [41, 42]. Oxidation products of 5mC have been identified in all mammalian tissues examined, albeit at low and variable levels. Thus, while 5mC is found in all tissues to correspond to approximately 5% of all cytosines, 5hmC has been reported to be present at approximately 0.5% of all cytosines in the CNS [43], 0.08% in embryonic stem cells (ESCs) [44] and 0.03%-0.06% in the spleen and testes [43]. The products of further oxidation have been less studied, but current estimates suggest 5fC and 5caC to be present at approximately 10-fold to 100-fold lower levels than 5hmC (reviewed in [43]). In broad agreement with this, kinetic studies of the TET catalytic activity indicate that the rate of 5hmC and 5fC oxidation is significantly reduced (approximately 5.6 fold and 10.2 fold respectively), compared to the initial rate of 5mC oxidation [37, 45], indicative of a lower substrate preference for the more oxidised cytosine modifications.

In addition to their roles in 5mC oxidation and turnover, TET proteins have also been shown to catalyse the oxidation of thymine bases in DNA to form 5-hydroxymethyluracil (5hmU) in ESCs, albeit in very low amounts [46]. The epigenetic significance of this modification, however, remains to be explored.

The three mammalian TET proteins have broadly similar structures. They all contain a C-terminal, core catalytic domain comprising a double-stranded β-helix (DSBH) adjacent to a cysteine-rich domain [47] (Figure 2). This catalytic domain alone has been shown to be able to localise to the

nucleus and oxidise 5mC [33, 48, 49]. The nuclear localisation of all 3 native TET proteins has been demonstrated, although there is also evidence of their translocation to the cytoplasm under some cellular conditions [50]. TET1 and TET3 also contain an N-terminal, DNA-targeting, CXXC domain [51]. By contrast, the putative CXXC domain of TET2 is encoded by a distinct gene, termed *Idax*, that has become separated from *Tet2* by a genomic inversion during evolution [47, 52]. The differential tissue- and developmental stage-specific patterns of the TET proteins, together with their physiological and pathophysiological functions, are increasingly becoming established (Table 1). Splice variants have also been identified for all three paralogues, including, in the case of TET1 and TET3, variants which lack the CXXC domain, and serve distinct functional roles [53-55].

Histone Demethylation by the Jumonji Family of 2-OGDD Proteins

Histone methylation, like DNA methylation, was thought to be a stable, covalent modification, until the discovery of enzymatic systems which can remove it. Two classes of enzymes, the lysine-specific demethylase (LSD) family and members of the Jumonji C (JmjC) family, are now recognised to effect histone demethylation by distinct mechanisms. The LSD family consist of FAD-dependent amine oxidases, which can remove both mono- and di-methyl histone lysine marks, but cannot demethylate tri-methyl lysine residues. (For a recent review of the LSD family, see [56]). The JmjC family are grouped on the basis of the presence of homology to a catalytic "jumonji" domain. Jumonji is a mouse mutation, named after the Japanese word for cross, which was identified in a gene trap screen and exhibited an abnormal, cross-shaped neural groove morphology [57]. The JmjC domain-containing proteins, like the TETs, are members of the 2-OGDD superfamily and were predicted and subsequently demonstrated to hydroxylate mono-, di- and tri-methylated lysine and arginine residues in N-terminal histone tails (reviewed in [20]; Figure 1B). There are currently over 30 proteins identified in humans to belong to the JmjC group and most (but not all) of these have been associated with histone demethylation (reviewed in [58]). They can be further classified into subfamilies, which typically may share substrate specificity, dependent upon the presence of other domains, including the TUDOR, CXXC, PHD, FBOX, ARID, LRR and JmjN domains (reviewed in[59]; Table 2). As is the case for the TET enzymes, specific physiological and pathophysiological roles are increasingly been identified for different JmjC demethylases, notably in development and certain cancers (reviewed in [60, 61]; Table 2).

HIF-PHD and FIH; Prototype Oxygen sensors?

Molecular oxygen (O_2) is an obligate substrate for all 2-OGDDs, which raises the intriguing possibility that these chromatin erasers may act as primary O_2 sensors in development, homeostasis and/or disease. The most highly studied members of the 2-OGDD superfamily, which are established to play a role in O_2-sensing, are the three closely-related hypoxia-inducible factor-1 prolyl hydroxylases (HIF-PHD1-3) [62]. These act to regulate the stability, and hence activity, of the hypoxia- (and other stress-) inducible transcription factor, HIF. Under well-oxygenated conditions, HIF-PHDs act to hydroxylate specific, conserved proline residues within the HIFα subunit (of which there are three homologues in higher metazoans). This creates a structural change in the protein that renders it subject to proteasomal degradation *via* binding to the von Hippel-Lindau (VHL) protein (a component of the ubiquitin ligase complex) [62]. Another 2-OGDD enzyme, termed factor-inhibiting-HIF (FIH), similarly utilises O_2 to hydroxylate a conserved asparagine residue within the HIFα subunit [63]. This results in a steric inhibition of the recruitment of the transcriptional coactivators, p300 and CBP, further inhibiting the activity of HIF under well-oxygenated conditions. Under hypoxia, the activities of both PHDs and FIH are compromised, allowing the HIF-dependent transcriptional

activation of a plethora of genes involved in processes involved in the homeostatic responses to hypoxia, including metabolism, angiogenesis and erythropoiesis [62].

However, for PHDs and FIH to be considered true oxygen sensors, their K_M values for oxygen (the concentrations of oxygen that support a half-maximal initial catalytic rate) must be such to allow for the modulation of their activities within ranges typically found in tissues (10-30μM). The studies performed to determine the K_Ms for O_2 of these enzymes have yielded differing results (Table 3). Currently, the consensus is that while PHDs are likely to be *bona-fide* direct O_2 sensors, at least in some cell and tissue types, the observed (low) K_M values of FIH preclude it from being considered a true O_2 sensor [62, 64].

Evidence for Regulation of TETs by [O_2]

It is an intriguing possibility that, like PHDs, the epigenetic modifying members of the 2-OGDD superfamily might also act, in some cases, as oxygen sensors. Individual TET proteins are increasingly being shown to play specific functional roles in embryonic development, both in the maintenance of pluripotency in ESCs, and in the acquisition of cellular fate [48, 65, 66]. In addition, the dysregulation of TETs has been associated with, (and in some cases has been suggested to be causal in), the development and progression of some pathologies, most notably cancer [67]. In both these physiological and pathological settings, the availability of molecular oxygen is known to be a critical determining factor.

The K_M values for O_2 of the purified catalytic domains of both TET1 and TET 2 have been investigated, and were found to be relatively equivalent, and of the order of 30μM [68]. This is considerably lower than values reported for the HIF-PHDs (90-250μM [69, 70]), and suggests that the TETs would retain significant activity under relatively hypoxic conditions. However, in a separate study, the effects of varying O_2 concentrations upon the activities of purified, recombinant TET1 and TET2 were determined. Significant reductions (45% and 52% for TET1 and TET2 respectively) were observed at 0.5% O_2 [71], suggesting that the activities of these enzymes *could* be modified by available [O_2], in some cellular environments. The reason for the discrepancy in these results is not clear, but may reflect the use of truncated, catalytic domains, compared to full-length proteins. The K_M of TET3 for O_2 has not been determined to date.

A reduction in TET activity and 5hmC levels is a hallmark of many tumours [72] and yet, perhaps significantly, mutations in the TET enzymes themselves have often not been identified [72]. This might reflect the activities of TETs becoming compromised in the more hypoxic, tumorigenic environment. Consistent with this, several human and mouse cancer cells, which were cultured under graded hypoxic conditions that were physiologically relevant to the tumour environment (down to 0.5% O_2), exhibited a dose- and time-dependent decrease in global 5hmC levels [71]. It should, however, be noted that the precise intracellular concentrations of O_2, achieved in such experiments, are difficult to assess and are likely to fluctuate due to cellular O_2 consumption and the rate of diffusion of O_2 through the media [73, 74]. These limitations similarly apply to the interpretation of other cell culture-based experiments described below. *In vivo*, the gene-expression patterns of tumours are known to reflect the hypoxic level of their environment [75] and have been used to assign them to hypoxic, normal or intermediate groups. Analyses of the methylation status of tumours demonstrated the hyper-(5mC)-methylated cluster to be predominantly associated with the more hypoxic tumours, whereas the low-methylated cluster were enriched for normoxic tumours [71]. The causal significance of [O_2] with regard to the methylation status of tumour tissue *in vivo* was also demonstrated in this study. Thus, increased hypoxia in mouse breast tumours was

shown to increase hypermethylation, while restoration of oxygenation to the tumour abrogated this effect. The inhibition of TET activity may, at least in part, account for these observations.

The importance of TETs in ESC differentiation and mammalian development are increasingly being demonstrated [48, 65, 66, 76-85]. Graded levels of O_2 are known to exist within the developing mammalian embryo [86-88], and it is intriguing to speculate that the differential activities of TETs, dependent upon their distinct microenvironments, might (in part) be a determinant of cellular fate during differentiation. We recently investigated the effects of the available [O_2], upon the activities of each of the three TET enzymes (as assessed by levels of 5hmC), when overexpressed in HEK-293T cells [44]. Our data demonstrated distinct patterns of inhibition of the three paralogues by [O_2] over a range (0.5-5%) that is physiologically relevant within the developing embryo [88, 89]. Thus, the activity of TET1 was unaltered in cells cultured at 5%, compared to atmospheric (~21%) O_2, but was significantly reduced upon culture at 3% O_2 and below. By contrast, the activity of TET2 remained unchanged at 3%, compared to atmospheric, O_2 (and was little inhibited at [O_2] as low as 0.5%). Surprisingly, we also found that the activity of TET3 was *increased* at 3%, relative to atmospheric O_2, but decreased again at levels below that [44]. We hypothesise that hypoxic culture conditions might result in changes in levels of TCA metabolites, which are known to affect the activity of 2-OGDDs. Accordingly, the levels of fumarate and succinate (metabolites known to inhibit TET proteins due to competitive steric inhibition of 2-OG) were found to be significantly decreased in HEK-293T cells cultured at 3%, compared to atmospheric, O_2.

The observations above therefore suggest that the activities of the TET proteins might be differentially-regulated by graded levels of O_2, and by levels of TCA metabolites, which themselves might be dependent upon O_2 availability, probably in a cell-type-specific manner. It can further be envisaged how the differential activities of the TET proteins might combine to generate distinct patterns of 5hmC levels within the developing embryo, dependent upon the (spatio-specific) [O_2] (Figure 3). Intriguingly, we also evidenced a transient burst of increased 5hmC during early mouse ESC differentiation that was both dependent upon TET1 (specifically), and inhibited by low (1%) [O_2]. The precise significance of these findings in regard to the acquisition of cell fate during development remains to be determined. However, they demonstrate the potential of the differential regulation of TET activities to effect the [O_2]-dependent establishment of distinct epigenetic cellular programmes during development.

In addition to the ability of [O_2] to regulate TET activity, multiple studies have demonstrated the transcriptional expression of TET enzymes to be dependent upon the cellular O_2 availability. Thus, increases in TET1/2 and 3 have all been reported in various cell types under hypoxic conditions [71, 90-94] which, in some cases, have been demonstrated to be dependent upon HIF1 [71, 91, 93]. As in the case of TET activity, the levels of expression of the different TET proteins show distinct patterns of sensitivity to different levels of hypoxia [93]. In addition, the expression of TET2 has also been reported to be inhibited in the glioblastoma cells line, A172, under more hypoxic cell culture conditions (by contrast to TET1 and TET3 whose expression was significantly increased) [94]. Thus, [O_2] may act differentially upon the TET proteins and in a cell-type specific manner to regulate their expression.

There is also evidence that the TET proteins participate functionally in the cellular hypoxic response, further strengthening the potential relationship between TET proteins and O_2-sensing mechanisms. For instance, in neuroblastoma cells, both TET1 function and increased levels of 5hmC were found to be causal in regulating the hypoxia-induced transcriptional program [95]. A subset of genomic locations which became enriched for 5hmC in hypoxia were found to be at, or near, HIF-1 binding sites, and to facilitate HIF binding. The functional involvement of TETs in a novel pathway by which

hypoxia promotes tumour malignancy has also been reported. Thus, in primary breast cancer cells, hypoxia induced the HIF1α-mediated expression of TET1 and TET3, together with a global increase in 5hmC. Further, TET1 and TET3 were shown to act co-ordinately to activate the TNFα-p38-MAPK signalling pathway and the acquisition of tumorigenic characteristics, *via* direct co-binding to the TNFα gene promoter [96].

Intriguingly, several studies have suggested that the transcriptional expression of the TETs themselves may further be regulated by hypoxia-induced changes in 5hmC (and thus potentially 5mC). Thus, the methylation status of both the *Tet1* and *Tet2* gene promoters in retinal pigment epithelial cells were found to become hypomethylated under conditions of chemically-induced hypoxia [90]. In addition, as stated above, we observed a transitory burst of 5hmC generation during early-differentiating ESCs that was dependent upon TET1 and was inhibited under low (1%) O_2 [44]. Strikingly, the major genomic target which became enriched for 5hmC in this system was shown to be *Tet3*. Given the association between TET3 expression and neural differentiation and function that is increasingly becoming elucidated [66, 97, 98], these observations may give further clues as to how gradients of [O_2] (known to be present in the early embryo) may act to determine cell fate, *via* regulation of TET functions (See Figure 4 for associations between [O_2] and TET function).

Regulation of JmjCs by Oxygen Availability

As in the case of the TET enzymes, the dysregulation of specific JmjC proteins (with known demethylase activity) has been shown to be associated with both developmental impairment and tumorigenesis; processes known to be dependent upon molecular oxygen availability (reviewed in [61, 99], see also Table 2). Moreover, many studies have demonstrated that exposure to hypoxia can alter the methylation status of histones and the epigenetic landscape in cultured cells (reviewed in [100]). Consistent with hypoxia acting to inhibit JmjC activity, increased H3K4me3, H3K9me2/me3 and H3K36me3 levels have all been reported under low [O_2] conditions [101-103]. In one case, this was attributed to the specific inhibition of KDM5A (JARID1A) [103]. Some *in vitro* kinetic studies have also demonstrated the dependence of the activities of specific JmjC members upon [O_2]. For example, the activity of recombinant, (pseudogene-encoded) KDM4E was shown to display a near-linear, graded response to O_2, over a physiologically-relevant concentration (0.5 to 20%) range. This study further demonstrated that the enzymatic activity was very dependent upon the methylation status of the substrate, since enhanced activity was observed upon a higher-methylated substrate [104]. More recently, the activity of KDM4A (both when expressed in cells, and as purified protein) was shown to demonstrate a graded reduction in response to depleting O_2 concentrations [105]. Also, as we observed for the TET activities, there is some evidence that JmjC enzymes might be differentially regulated by levels of O_2. Thus, when overexpressed in HeLa cells, KDM3A (JMJD1A) activity was maintained under 0.2% O_2, while that of KDM4B (JMJD2B) was attenuated [106].

While the K_M values for the JmjC proteins have proven technically challenging to evaluate, some estimates have been made (Table 3). These data also indicate the differential sensitivity of JmjCs towards reduced O_2 levels, and suggest the potential for some, but likely not all, to be sensitive to physiological changes in [O_2]. This differential regulation of activity by oxygen may be of particular functional significance during embryonic development. Genes that are poised for activation (as required during subsequent differentiation) are marked with both positive (H3K4me3) and repressive (H3K27me3) histone methylation marks. This bivalent state is both established and resolved by Polycomb and Trithorax group complexes, which can recruit JmjCs, as appropriate, to remove the activating or repressive mark [107]. Clearly, the differential sensitivities of these

activities to O_2 could act to determine which sets of genes were activated or repressed, dependent upon their spatial position and local O_2 microenvironment.

Another mechanism whereby hypoxia has been shown to regulate the activities of the JmjC proteins in mammalian cells is *via* induction of the enzymatic reduction of 2-OG to generate the "oncometabolite" D-2-hydroxyglutarate (D-2HG). D2HG is a competitive inhibitor of 2-OGDDs, including JmjCs, and has been shown in various cell types to act to increase methylation marks, including H3K9me3, under hypoxic conditions [108].

In addition, as was found to be the case for DNA methylation, the relationship between hypoxia, JmjC function and histone methylation is complex. Many JmjC proteins are known to be direct transcriptional targets of HIF and therefore their expression levels increase under more hypoxic conditions. These include KDM3A, KDM4B, KDM4C, KDM5B, and KDM5C [106, 109-113]. Moreover, the expression of some other JmjC proteins have also been shown to increase in hypoxia, although they have not been identified as direct HIF targets, suggesting the contribution of additional mechanism(s) [58, 114, 115]. Again, as was found to be the case for the TETs, there is evidence for the involvement of JmjCs in the functional cellular response to hypoxia. Thus, under hypoxia, the increased expression of KDM3A promotes its binding at HRE sites within the regulatory regions of the HIF target genes. This then promotes demethylation of the (repressive) H3K9me2/me3 mark and induction of gene expression [116]. A direct interaction between HIF1α and (increased levels of) KDM4C has also been observed in hypoxia. In this case it has been proposed that HIF1α is responsible for guiding KDM4C to its target promoters, again to enhance the demethylation of the H3K9me2/me3 mark and upregulate gene expression [117]. This has been demonstrated to be of potential functional significance in tumorigenesis, since ablation of KDM4C in mice acted to inhibit breast tumour growth and lung metastasis. The composite relationships between hypoxia, JmjC activity, expression and target specificity will therefore underlie distinct cell type- and locus-specific changes in histone methylation patterns in response to hypoxia.

Regulation of 2-OGDD Proteins by Redox-Dependent Mechanisms

The catalytic domain of 2-OGDDs is remarkably well-conserved across proteins of diverse functions [118]. All comprise a core structural motif consisting of eight β-strands arranged in a "jelly roll" or double-stranded β-helix, which is surrounded by α-helices[119]. Within this jelly roll a non-heme, ferrous (Fe^{2+}) iron is co-ordinated by a triad of two histidines and aspartate/glutamate. The remaining three co-ordination sites of the iron are only loosely occupied by labile water molecules [119]. This relatively low affinity of 2-OGDDs for iron renders the activities of the enzymes potentially susceptible to changes in cellular iron concentrations. In addition, it also renders the ferrous ion itself vulnerable to oxidation to the ferric (Fe3+) state, which is known to be inhibitory to 2-OGDD activity [120]. However, it should be noted that despite this co-ordination chemistry predicting a labile arrangement, the apparent K_M values of, for instance, the HIF-PHDs for Fe^{2+} are surprisingly low (Table 3). It is currently not known, however, whether the Fe^{3+} generated during the catalytic cycle (see below) binds as strongly as Fe^{2+}.

In the catalytic cycle, 2-OG and the target substrate to be hydroxylated first bind within the active site, displacing a co-ordinating water molecule. The binding of molecular oxygen to the ferrous iron then results in the splitting of the oxygen molecule. One oxygen atom effects oxidative decarboxylation of 2-OG to form succinate and CO_2, while the other is used in the generation of a highly-reactive ferryl-oxo species that acts to hydroxylate the target substrate [121]. In this process, Fe^{3+} is generated at the active site and needs to be reduced back to Fe^{2+} to maintain the activity of the 2-OGDD enzyme. Ascorbate is a necessary co-factor in this process and although the precise

mechanism is not fully understood, the role of ascorbate in maintaining the function of 2-OGDDs via the recycling of Fe^{3+} to Fe^{2+} is well established [122]. In the case of the HIF-PHDs, the K_M values for ascorbate have been determined and were found to be relatively high (Table 3). Accordingly, their activities have been demonstrated to be highly susceptible to physiological cellular fluctuations [123].

The susceptibility of the ferrous iron to oxidation (for example, by superoxide, hydrogen peroxide or peroxynitrite, amongst other ROS) might predict the activities of 2-OGDDs to be subject to inhibition by some ROS-generating processes. In addition, since ascorbate levels can fluctuate, dependent upon the intracellular glutathione:oxidised glutathione redox couple, the cellular redox state itself might act as a functional regulator of 2-OGDD activity.

Again, using the example of the HIF-PHDs, there is good evidence that ROS and/or cellular redox are regulators of the activities of 2-OGDD enzymes. However, the source and type of ROS, together with the mechanism(s) of inhibition have remained controversial. ROS production necessarily involves oxygen, and it is therefore not surprising that different concentrations (and forms) of ROS are generated under normoxia, hypoxia and anoxia. Opinion remains divided as to whether ROS levels increase or decrease during hypoxia (reviewed in [124]), and this may reflect the different levels of hypoxia imposed in the different experimental conditions applied. Thus, the role of ROS in mediating the hypoxic response and promoting HIF stabilisation and activity has been difficult to elucidate. Evidence for the involvement of both mitochondrial- and NADPH oxidase-derived ROS have been reported [125-130], but complete validation of the importance of either (or both) will probably await clarification of the precise ROS-dependent mechanism of inhibition of the HIF-PHDs. H_2O_2 is the ROS most commonly demonstrated to be functional in the stabilisation of HIF [131-133]. Thus, administration of H_2O_2 to cells has been shown to be sufficient to stabilise and activate HIF, even during normoxia [125, 130]. However, both superoxide and peroxynitrite have also been shown to mediate HIF1α stabilisation [134-137]. It should also be noted that (potentially cell-type-specific) redox-dependent mechanisms, other than those affecting the activity of PHDs, also impact upon the regulation of HIF signalling. These include the redox-sensitive regulation of transcription [138-140] and/or the direct oxidation of reactive cysteine residues of the HIF subunits themselves (for excellent reviews, see [141, 142]) While clearly not the focus of this article, these mechanisms need to be taken into consideration in the wider context of cellular redox/ROS generation and the regulation of HIF-signalling.

As stated above, the absolute requirement for Fe^{2+} and its susceptibility to direct oxidation, notably by H_2O_2, might underlie the ROS-dependent regulation of HIF-PHDs and potentially other 2-OGDDs. These changes in ROS might themselves be effected or exacerbated by hypoxia and might therefore (in the case of the HIF-PHDs) promote the hypoxic activation of HIF, or might act independently. Oxidation of Fe^{2+} might be mediated by the targeted (and potentially highly regulated) generation of ROS, for instance by NADPH oxidases and/or mitochondria (reviewed in [143]). Alternatively, a pro-oxidant change in the (global) intra-cellular redox state (notably resulting in a reduction in the reduced:oxidised glutathione ratio; GSH:GSSG,) might recruit ascorbate as an intracellular free radical scavenger, thereby preventing its ability to reduce ferric iron and maintain HIF-PHD activity (Figure 5). Consistent with this mechanism of [Fe^{2+}]-dependent HIF-PHD regulation, the transcription factor JunD was shown in mouse fibroblasts (3T3 cells) to upregulate antioxidant defence genes to prevent the accumulation of H_2O_2. The genetic ablation of JunD consequently resulted in elevated levels of H_2O_2 and inactivation of PHD2 *via* oxidation of Fe^{2+} within its catalytic domain [132]. There are, in addition, other mechanisms which might also be involved in the redox-mediated regulation of HIF-PHD activity and some evidence for these has been reported. The modulation of function by

H_2O_2-mediated oxidation of susceptible cysteine residues has been well-documented for protein tyrosine kinases and phosphatases [144], and is believed to be an important mechanism in many redox-mediated signalling pathways. Disulphide bond-mediated homo-dimerization and inactivation of PHD-1, -2 and -3 have been observed in cancer cells upon administration of H_2O_2, and oxidation of specific cysteine residues within the catalytic protein domain(s) were implicated in this dimerization [145]. However, it remains unclear whether this dimerization can occur under physiological cellular conditions. The redox-mediated initiation of a signal transduction cascade, leading to the post-translational modification (and inhibition) of HIF-PHDs, is a further potential mechanism that might manifest as ROS-dependent regulation of activity. In support of this, the p38 MAPK signalling pathway has been shown to mediate mitochondrial-generated, ROS-dependent activation of HIF-1 under hypoxic conditions [146]. However, it is not clear in this case whether the signalling cascade might target the HIF-PHD(s) or HIF1α itself. Finally, it is known that concentrations of both the obligatory (2-OG) and inhibitory (succinate and fumarate) metabolites of 2-OGDDs might also be altered by free radical production associated with mitochondrial dysfunction [147] and this might serve further to mediate the observed ROS-dependent regulation of HIF-PHDs (Figure 5).

Redox-dependent Regulation of TET Enzymes.

As in the case of the regulation of TET activity by O_2 availability, only a few studies have investigated the direct regulation of TET activity by redox mechanisms. Consistent with their requirement for Fe^{2+}, ascorbate administration to various cultured cells over the range of 1μM-1mM was shown to increase the global levels of 5hmC rapidly [148] and this increase was demonstrated to be inhibited by siRNAs targeted to (all 3) TETs. In another study, treatment of epithelial cells with 150μM H_2O_2 for 3 days resulted in a lowered (locus-specific) level of genomic 5hmC and reduced TET activity, although the mechanism underlying this inhibition was not determined [149].

Environmental stresses (such as toxic chemicals, heavy metals, air particles and ozone) are all known to induce the production of pro-oxidants [150]. Therefore their effects upon the activities of TETs may be significant in pathological epigenetic dysregulation and are beginning to be explored. For example, exposure of HEK293 cells to the ROS-generating, benzene metabolite hydroquinone, was found to result in a more oxidative cellular redox state which correlated, perhaps surprisingly, with *increased* nuclear TET activity and higher nuclear levels of TET1 protein, with no change in TET1 mRNA levels [151]. The authors suggest that hydroquinone exposure might induce redox-dependent changes in the nuclear import/export mechanisms of TET1. Alternatively, redox-regulated post-translational modifications affecting TET1 activity and or stability may underlie the observations. Redox-active quinones were similarly found to promote the conversion of 5mC to 5hmC, in a TET-dependent fashion, in a variety of cell lines, including mouse ESCs [152]. In this case, the increases in 5hmC upon quinone administration were shown to correlate with increased expression (at both mRNA and protein level) of the labile, iron-sensitive ferritin light-chain protein which could be blocked by chelation of labile iron. The potential regulation of TET activity by modulation of the labile iron pool may have particular significance with regard to cancer development and therapy [153].

Very recently, the post translational phosphorylation of TET2 at serine 99, mediated by the (potentially redox-regulated) AMPK-signalling pathway [154], was demonstrated to increase TET2 stability and hence activity [155]. Thus the redox-dependent regulation of TET stability and activity by post-translational modifications and other potential mechanisms will likely prove an important area of future investigation. An understanding of the source, type and localisation of the ROS signal will be important considerations in the experimental design of these investigations.

Functional Consequences of Redox-modulation of TETs.

The availability of ascorbate, and its role in the maintenance of the reduced ferrous iron within the active sites of TETs, may impact upon many important cellular functions and processes. Cellular reprogramming, within the early mammalian embryo and in primordial germ cells, involves phases of global DNA demethylation [156, 157] which are likely to be dependent upon the activity of TET enzymes. Further, the addition of vitamin C to mouse ESCs cultured *in vitro,* was shown to promote a rapid, global increase in levels of 5hmC, that was completely dependent upon TET1 and TET2 [158], highlighting the importance of vitamin C in the maintenance of TET activity and DNA methylation fidelity in ESCs. The functional significance of these observations, with respect to a potential role of regulated vitamin C levels in developmental-reprogramming *in vivo,* remain to be investigated. The levels of ascorbate have also been shown to mediate somatic cell reprogramming by acting as a switch in the regulation of TET1 function [159]. In a recent study, vitamin C administration was also shown to promote the maturation and developmental competence of pig oocytes, concomitant with global DNA demethylation, although the requirement for a specific TET was not demonstrated in this process [160]. It is also perhaps noteworthy that the levels of expression of the TET enzymes were found to be differentially affected in these experiments. Thus TET2 (mRNA and protein) levels were significantly increased, while mRNA levels of TET3 were decreased, upon vitamin C administration. (The redox-dependent mechanisms underlying the apparent regulation of *expression* of TET enzymes remain to be explored). In another study, administration of ascorbate was demonstrated to inhibit the expression and secretion of apo(a) (a liver-expressed glycoprotein found in plasma and a risk factor for coronary heart disease) in HepG2 cells, *via* promoting TET2-dependent DNA demethylation [161].

A potential functional consequence of the redox-dependent regulation of TET activity is also suggested by the association of traffic-related air pollution, (which is well known to promote oxidative cellular damage [162]), with altered (increased) levels of global 5hmC levels, found in the airway epithelial cells of asthma sufferers [163]. In addition, the redox-dependent up-regulation of TET1 expression and function has been reported to underlie the acquisition of resistance to the anticancer agent, 5-fluorouracil [164].

Regulation of JmjCs by Redox Mechanisms

As in the case of the TETs, ascorbate has been shown to be an essential cofactor in the maintenance of the activities of the JmjC histone demethylases, evidencing their requirement for Fe^{2+} (reviewed in [165]). In one study, an increase in several histone methylation marks, including H3K4me3, was exhibited after human bronchial epithelial cells (BAEC-2Bs) were exposed to H_2O_2 for 3 hours, while pre-incubation with ascorbate prevented these changes [149]. This suggests that the direct of oxidation of Fe^{2+} by H_2O_2, which is reversible by ascorbate, was the cause of the inhibition in this case. There have also been several studies investigating the potential inhibition of the histone demethylases by metal ions which can compete with Fe(II) for the active site and/or induce oxidative stress. Nickel exposure has been shown to increase global levels of H3K4me3, H3K9me2 and H3K9me1 in several studies [166, 167]. Mechanistically, this is probably due to the displacement of the Fe(II) by nickel, which has an approximately 3-times greater affinity for the JmjC active site than iron itself (reviewed in [168]). Moreover, in a number of cell-based studies, both arsenic and chromium exposure have been shown to result in altered patterns of histone methylation, potentially mediated by dysregulated JmjC activity [167, 169-171]. Arsenic and chromium are both potent generators of oxidative stress within cells, but the precise mechanisms whereby they might inhibit 2-OGDDs is not clear [168]. Intriguingly, in one study the dysregulation of histone

methylation by chromium was shown to be (in part) ablated by ascorbate, suggesting that chromium might be acting to deplete the reduced intracellular levels of ascorbate [170].

As noted above, there is evidence for the redox-dependent regulation of HIF-PHDs by cysteine-thiol oxidation, leading to disulphide bond formation and protein inactivation [145]. As yet, this mechanism has not been demonstrated to be functionally relevant with respect to either JmjCs or TETs. However, such a mechanism has been suggested to switch the activity of a member of the LSD histone demethylase; KDM1A [172]. Indeed, the involvement of redox-mechanisms are increasingly being demonstrated and/or suggested to underlie regulation of many epigenetic-modifying processes, in addition to the demethylation of DNA and histones [173].

Functional Consequences of Redox Regulation of JmjCs

As is the case for the TET enzymes, the JmjC histone demethylases appear to play important roles in cellular programming and reprogramming. The reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) is known to be greatly accelerated and enhanced by ascorbate. It has also been demonstrated that this is dependent upon induction of H3K36me2/3 demethylation, catalysed by KDM2A and KDM2B (JHDM1A and JHDM1B) [174]. Intriguingly, in that study it was also noted that KDM2B co-operates with Oct4 (perhaps through a physical interaction) to induce expression of microRNA cluster 302/367, which is an integral component of the pluripotent phenotype. In another study, ascorbic acid was demonstrated to promote the maturation of porcine oocytes, concomitant with the erasure of the (repressive) histone methylation mark, H3K27me3. The levels of other histone methylation marks assessed in this study either did not change (H3K9me3), or were shown to increase (H3K4me3 and H3K36me3). Intriguingly, the mRNA expression of KMD5B (which acts to remove the activating H3K4me3 mark) was decreased by ascorbate administration, perhaps consistent with the increase in H3K4me3 levels. The potential mechanisms underlying this ascorbate- (redox)-mediated regulation of transcription of the JmjC(s) remain to be investigated. Given the clear association between epigenetic dysregulation in cancers and the capability of ascorbate to regulate JmjCs (and TETs), it would seem highly important to understand such mechanisms fully, in order to exploit the therapeutic possibilities of ascorbate.

The dysregulation of the JmjCs by exposure to toxic metals, such as nickel, arsenic and chromium, probably accounts in part for their toxicity. For example, exposure to these elements has been implicated in the occurrence of numerous human cancers and in many cases this has been attributed to their abilities to alter chromatin structure (reviewed in [168]). A full understanding of the redox-based mechanisms that underlie the dysregulation of these epigenetic modifiers, in response to such environmental stresses, could have major beneficial future therapeutic implications.

Regulation of RNA Function by Methylation.

Although less studied, it is clear that the function of RNA is also subject to regulation by covalent methylation, predominantly at N^6-methyladenosine (m^6A) (reviewed in [175]). Further, the discovery of enzymatic systems capable of both adding and removing m^6A has led to the proposal that they too may be subject to dynamic regulation by epigenetic mechanisms (termed “RNA epigenetics” (reviewed in [176]). Intriguingly, the demethylating enzymatic systems have again proven to be members of the 2-OGDD family, which, as in the case of the TETs, act to remove methyl groups by successive oxidative reactions [176, 177]. Fat mass and obesity-associated protein (FTO) was the first RNA demethylase to be identified [178]. It preferentially acts upon single-stranded RNA or DNA to demethylate m^6A, and has been shown to be nuclear-localised. This observation, together with the very low levels of m^6A on genomic DNA within (most) mammalian cells and tissues, suggest that its

main target substrate is nuclear RNA (including mRNA, lucRNA and potentially other RNA species). Little is known of the functional consequences of FTO-mediated RNA demethylation. However, genetic ablation of FTO in mice was shown to increase post-natal lethality and growth retardation [179], while the levels of FTO expression are regulated by feeding and fasting, consistent with an association with obesity [180].

ALKBH5 is another mammalian demethylase, shown to be a member of the 2-OGDD superfamily, which acts to reverse m^6A by oxidation in mRNA both *in vitro* and *in vivo* [181]. As for FTO, ALKBH5 has also been shown to co-localize with nuclear speckles and to show efficient demethylation activity towards various nuclear RNA species, including mRNA [181]. Silencing of ALKBH5 in human cell lines acted to both increase total m^6A levels on polyadenylated RNA and significantly affected mRNA nuclear export and processing [181]. In mice, ALKBH5 has been shown to display the highest expression levels in testes. Intriguingly, ALKBH5-deficient male mice were characterised by impaired fertility, resulting from apoptosis of meiotic metaphase-stage spermatocytes [181]. Clearly, as members of the Fe(II)-dependent 2-OGDD superfamily, both of these RNA demethylases may be subject to regulation by both O_2 availability and redox-dependent mechanisms. This is a completely unexplored area of investigation which will, no doubt, be the subject of future investigations.

Conclusions

The finding that epigenetic erasers, which act to remove methyl groups from DNA and protein, are members of the 2-OGDD superfamily has clear implications with respect to dynamic regulatory mechanisms which might modulate their functions. The capacity for the activities of the TET and JmjC proteins to be regulated by metabolites, oxygen availability and redox-based mechanisms are likely critical in their proven functions in cellular fate determination during development. It might also underlie their dysregulation in pathologies associated with distortions of metabolic flux, hypoxia and oxidative stress, such as tumorigenesis. The wider clinical consequences of such regulation are also clear. In addition to cancer, many other pathological conditions, ranging from heart failure to stroke, can result in lasting oxygen shortage associated with worsening morbidity and mortality. The impaired activity of the epigenetic modifiers in these clinical settings suggest a clear potential mechanism whereby an increasingly hypoxic environment might lead to altered gene expression, contributing to disease progression.

Definitive evidence for physiological [O_2]-dependent modulation of activity of both TETs and JmjCs is now growing. Further, the members of both families of enzymes have, in some studies, demonstrated differential dependencies upon O_2 availability. This clearly might facilitate a great range of cellular responses to a given environmental cue, dependent upon the expression patterns of the enzymes in any given cell type. Similarly, there is increasing evidence for redox regulation of these enzymes, although the precise mechanisms remain to be determined. However, it is clear that the relationships between both hypoxia/cellular redox and TET/JmjC activity are complex. For example, reductions in specific activity due to lower available O_2 and/or increased ROS, can, in some instances, be countered by induced gene expression, often making elucidation of the underlying mechanisms challenging.

The roles of TETs and JmjCs in (dynamic) cellular homeostatic processes have not been extensively studied. However, TET2 specifically has been implicated as both a master regulator of smooth muscle cell plasticity [182] and as a controller of cardiomyocyte gene expression during the hypertrophic response [183]. JmjCs have similarly been implicated in cardiac hypertrophy [184] and also in the maintenance of cellular redox homeostasis [185]. The potential roles of these proteins in

other homeostatic mechanisms in which O_2 and/or cellular redox are important regulatory cues (such as angiogenesis and erythropoiesis), remain to be explored.

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Table 1. Expression profiles, and (patho)- physiological functions of TET proteins

TET Protein	Expression as described in the Human Protein Atlas [186, 187]	Functions (biological and catalytic)	Associations with Disease
TET 1 (Methylcytosine dioxygenase 1)	RNA expression in cerebral cortex (neuronal, glial and endothelial cells), thyroid and parathyroid glands (glandular and endothelial cells), smooth muscle cells, gallbladder, male and female reproductive tracts	Active DNA demethylation by catalytic conversion of 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) [33] Chromatin regulation by O-linked N-acetylglucosamine transferase recruitment to transcriptional start sites promoting histone H2B GlcNAcylation [188] Involved in trophoblast stem cell maintenance and integrity, and cell-cycle progression[189] Transcriptional regulation *via* interaction with the transcriptional corepressor, SIN3A [190]	Upregulated in inflammatory microenvironment of hyperinsulinemia and invasive ductal carcinoma [191]. Plays an essential oncogenic role in MLL-rearranged leukaemia [192] Downregulated in gastric and prostate cancer and numerous solid cancers (breast, melanoma, glioma, colon and liver) along with other TET family members [96, 193-196].
TET 2 (Methylcytosine dioxygenase 2)	RNA and protein expressed in cerebral cortex, myocytes, smooth muscle cells, cells of the immune system (bone marrow, appendix, tonsils, spleen), thyroid, parathyroid and adrenal gland, hepatocytes and bile duct cells, islet of Langerhans and exocrine glandular cells of pancreas, the gastrointestinal tract, adipocytes, male and female reproductive organs and skin.	Shared demethylation and chromatin regulation role with other TET family members [36, 37, 48, 190] Regulates hematopoietic stem cell (HSC) self-renewal, haematopoiesis and myeloid lineage commitment and differentiation.[197] TET2 is targeted by microRNA-22 to regulate HSC self-renewal and transformation [198] Involved in trophoblast stem cell maintenance and integrity [189]	Somatic TET2 mutations altering the enzymes catalytic activity observed in many cancers (myelodysplasia, AML, breast, lung, pancreas and liver [52, 196, 199-201]) 4q24 (*Tet2*) is a common chromosomal deletion observed in hematopoietic malignancies. [202] TET2 mutations are frequently observed in chronic myelomonocytic leukaemia patients and are used as a prognostic biomarker for the disease [203]
TET 3 (Methylcytosine dioxygenase 3)	Expression profile similar to TET2 at RNA and protein level	Shared demethylation and chromatin regulation role with other TET family members [36, 37, 48, 190] Involved in developmental regulation *via* chromatin reprograming in zygote following fertilization, as well as transcriptional regulation of early development genes [204] Responsible for global erasure of DNA methylation patterns in the paternal pronucleus in zygotes [205]. Stabilizes thyroid hormone T3 nuclear receptors by promoting their association to chromatin [206].	Negative regulator of IFNβ in response to viral infection.[207] Loss of function results in increased aggressive myeloid cancer in mice [208]

		Regulates the balance between neural and mesoderm cell fate determination [66].	

Table 2. Functions of JmjC histone demethylases

JmjC	Alternative names	Functional Domains	Histone substrate	Function	Role in Disease
KDM2A	JHDM1A/ FBXL11, FBL11/FBL7/ CXXC8	JmjC; CXXC; PHD; FBox;LRR	$H3K6_{me1/2}$	Regulator of NF-KB pathway [209]. Integration of histone and DNA modifications *via* interactions with HP1. [210]	Oncogene in breast canc Promotes DNA methylatic through TET2 inhibition [211]
KDM2B	JHDM1B/FBXL10 /PCCX2/ CXXC2	JmjC; CXXC; PHD; FBox; LRR	$H3K6_{me1/2}$; $H3K4_{me3}$	Regulates somatic reprogramming through interaction with Polycomb complexes [212]. Regulator of apoptotic pathway [213]	Drives pancreatic cancer tumorigenicity through Polycomb interaction [214 Promotes breast cancer proliferation [215]
KDM3A	JHDM2A/JMJD1/ JMJD1A/ TSGA	JmjC	$H3K9_{me1/2}$	Direct target of HIF and upregulated in hypoxia [100]. Involved in cell cycle control[216]	Transcriptional regulation androgen receptors in prostate cancer [217]. Regulates ovarian stemness cancer chemoresistance [218]. Promotes cancer progression *via* HOXA1 gene regulation [216]
KDM3B	JMJD1B/NET22/5 qNCA	JmjC	$H3K9_{me1}$	Tumor-suppressive activity [219] Regulates somatic growth and female reproductive function [220]	Tumour–promoting activi [219]. Promotes colorect cancer progression [221] Promotes leukemogenes [222]
KDM4A	JMJD1C/TRIP8/J HDM2C	JmjN; JmjC; PHD; Tudor	$H3K9_{me2/3;}$ $H3K36_{me2/3}$	Regulates HIF-1 levels *via* the $H3K9_{me3}$ mark [223]	Deregulated in prostate, bladder, colorectal, lung and breast cancer [224]. Regulates tumour metabolism through interaction with E2F1 [225
KDM4B	JMJD2B/TDRD14 B	JmjN; JmjC; PHD; Tudor	$H3K9_{me2/3;}$ $H3K36_{me2/3}$	Direct target of HIF and upregulated in hypoxia [100] Serves as a DNA Damage Response protein [226]	Regulates estrogen receptor signalling casca in breast cancer [227]. Regulates peritoneal seeding of cancer cells [228]

KDM4C	TDRD14C/JHDM 3C/JMJD2C	JmjN; JmjC; PHD; Tudor	H3K9$_{me2/3;}$ H3K36$_{me2/3}$	Direct target of HIF and upregulated in hypoxia [100]	Involved in cell proliferatio and chromosome segregation in breast cancer [229]. In colon cancer mediates Wnt/Not pathway cross talk to regulate sphere formation in colon cancer [230]
KDM4D	JMJD2D/JHDM3 D	JmjN; JmjC	H3K9$_{me2/3;}$ H3K36$_{me2/3}$	Regulates DNA replication by facilitating pre-initiative complex formation [231]. Plays a role in DNA damage response [232]	Upregulated in pancreatic adenocarcinoma [233]
KDM4E	KDM4DL/JMJD2 D	JmjN; JmjC	(H3K4$_{me2/3}$	Putative pseudogene, but activity shown to regulated by O_2 availability in vitro [104]	
KDM5A	RBP2/JARID1A/R BBP2	JmjN; ARID; JmjC; PHD; C5HC2-ZF	H3K4$_{me2/3}$	Involved in maintenance of 3'UTR length through DICER1 [234].	Implicated in renal, ovaria liver and acute lymphoblastic leukemia (ALL). [235-238]
KDM5B	JARID1B/PUT1/P LU-1/CT31	JmjN; ARID; JmjC; PHD; C5HC2-ZF	H3K4$_{me2/3}$	Direct target of HIF and upregulated in hypoxia [100] Involved in maintenance of 3'UTR length through DICER1 [234].	Plays a role in melanoma development and progression [239]. Implicated in oral cancer and glioma [240, 241].
KDM5C	JAIRD1C/SMCX/ XE169/ DXS1272E/MRX1 3	JmjN; ARID; JmjC; PHD; C5HC2-ZF	H3K4$_{me2/3}$	Direct target of HIF and upregulated in hypoxia [100]	Plays a role in gastric cancer cell proliferation [242]. Overexpressed in breast cancer [243]
KDM5D	JARID1D/SMCY/ HYA	JmjN; ARID; JmjC; PHD; C5HC2-ZF	H3K4$_{me2/3}$	Evidence for a role in immune recognition [244]	Strong evidence for a role in prostate cancer [245]
KDM6A	UTX/ BA386N14.2	TPR; JmjC;	H3K27$_{me2/3}$	Regulates endoderm differentiation through WNT signalling pathway [246]	Mutated in bladder cance [247]. Mutations associate with Kabuki syndrome [24
KDM6B	JMJD3	JmjC;	H3K27$_{me2/3}$	Regulates endoderm differentiation through WNT signalling pathway [246] Involved in differentiation of germinal centre B-cells [249]	Mediate multiple myeloma cell growth and survival [250]. Promotes survival o diffuse large B-cell lymphoma subtypes [249]
KDM7A	JHDM1D	JmjC;PHD	H3K27$_{me1/2;}$ H3K9$_{me1/2;}$	Implicated in neural differentiation [251] Modulates endothelial inflammatory response [252]	Acts as a tumour growth suppressor role *via* downregulation of angiogenesis [253].
KDM7B	PHF8/JHDM1F/Z NF422	JmjC;PHD	H3K9$_{me1/2;}$ H4K20$_{me1}$	Regulates transcription of rRNAs [254, 255]	Promotes ALL, breast, lur and prostate cancer. [256 259]
KDM7C	PHF2/CENP-35/JHDM1E	JmjC;PHD	H3K9$_{me2}$	Regulates transcription of rRNAs [254]	Mutations associate with gastric and colorectal

					cancer [260] Downregulated in ALL [261] Tumour suppressor role i cancer development in association with p53 [262
KDM8	JMJD5ROX/MDIG	JmjC	$H3K36_{me2}$	Involved in a control of cell cycle and proliferation [263]	Implicated as a potential oncogene in colon cancer [264]
MINA	MDIG/RIOX2	JmjC	$H3K9_{me3}$	Implicated in DNA double strand break repair [265]	Contributes to pathogenesis of cancer b affecting cell growth and motility [266]. Prognostic marker of pancreatic cancer [267] Controls proliferation and invasion non-small cell lung cance and glioblastoma[268, 26
NO66	MAPJD/ROX	JmjC	$H3K4_{me1/3;}$ $H3K36_{me2}$	Potential role in ribosome biogenesis [270] Possible role in regulation of osteoblast-specific transcription [271]	Prognostic biomarker for colorectal cancer [272] Upregulated in renal cell tumours [273]

Domain Key : ARID – AT rich interacting domain; PHD- Plant Homeodomain; FBOX- FBox domain; CW- CW type zinc finger domain; TUDOR- Tudor domain; JmjC- Jumonji C domain; JmjN- Jumonji N domain; CXXC- CXXC zinc finger domain; TPR – tetratricopeptide domain

Table 3. K_M values for selected 2-OGDDs for O_2, 2-oxoglutarate, Fe^{2+} and ascorbic acid

Enzyme	$K_m^{app}(O_2)$/μM	K_m^{app}(2-OG)/μM	$K_m^{app}(Fe^{2+})$/μM	K_m^{app} (Ascorbic Acid)/μM
HIF-PHD1	230 [69]	2 ± 0.4 [274]	0.03 [122]	170 [70]
HIF-PHD2	~250 μM to 1.7 mM [69, 275-277]	1 ± 0.2 [274]	0.03 [122]	180 [70]
HIF-PHD3	230 [69]	12 ± 4 [274]	0.1 [122]	140 [70]
FIH	90-200 [278] 90 [70]	110 ± 20 μM [278] 25 ± 3 [70]	0.5 ± 0.2 [70]	260 ± 50 [70]
KDM4A $_{1-359}$	173 +23 [105] 57 ± 10 [279]	23 [280] 10-37 [279]	Not determined	Not determined
KDM4C	158 ± 13 [279]	17.1 ± 0.9 [281] 10-37 [279]	1.3 ± 0.4 μM [281]	Not determined
KDM4E	197 ± 16 [279]	10-37 [279]	Not determined	Not determined
TET1 $_{1367–2039}$	~30 [68]	55 [280]	5 [280]	Not determined
TET2 $_{916–1921}$	~30 [68]	60 [280]	4 [280]	Not determined

References

[1] Woodcock, C. L.; Dimitrov, S. Higher-order structure of chromatin and chromosomes. *Curr Opin Genet Dev* **11:**130-135; 2001.

[2] Grewal, S. I.; Moazed, D. Heterochromatin and epigenetic control of gene expression. *Science* **301:**798-802; 2003.

[3] Filion, G. J.; van Bemmel, J. G.; Braunschweig, U.; Talhout, W.; Kind, J.; Ward, L. D.; Brugman, W.; de Castro, I. J.; Kerkhoven, R. M.; Bussemaker, H. J.; van Steensel, B. Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. *Cell* **143:**212-224; 2010.

[4] Lleres, D.; James, J.; Swift, S.; Norman, D. G.; Lamond, A. I. Quantitative analysis of chromatin compaction in living cells using FLIM-FRET. *J Cell Biol* **187:**481-496; 2009.

[5] Swygert, S. G.; Peterson, C. L. Chromatin dynamics: interplay between remodeling enzymes and histone modifications. *Biochim Biophys Acta* **1839:**728-736; 2014.

[6] Kietzmann, T.; Petry, A.; Shvetsova, A.; Gerhold, J. M.; Gorlach, A. The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. *Br J Pharmacol* **174:**1533-1554; 2017.

[7] Jones, P. A.; Baylin, S. B. The epigenomics of cancer. *Cell* **128:**683-692; 2007.

[8] Talbert, P. B.; Henikoff, S. Histone variants--ancient wrap artists of the epigenome. *Nat Rev Mol Cell Biol* **11:**264-275; 2010.

[9] Hargreaves, D. C.; Crabtree, G. R. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res* **21:**396-420; 2011.

[10] Bird, A. DNA methylation patterns and epigenetic memory. *Genes Dev* **16:**6-21; 2002.

[11] Lister, R.; Pelizzola, M.; Dowen, R. H.; Hawkins, R. D.; Hon, G.; Tonti-Filippini, J.; Nery, J. R.; Lee, L.; Ye, Z.; Ngo, Q. M.; Edsall, L.; Antosiewicz-Bourget, J.; Stewart, R.; Ruotti, V.; Millar, A. H.; Thomson, J. A.; Ren, B.; Ecker, J. R. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462:**315-322; 2009.

[12] Hon, G. C.; Rajagopal, N.; Shen, Y.; McCleary, D. F.; Yue, F.; Dang, M. D.; Ren, B. Epigenetic memory at embryonic enhancers identified in DNA methylation maps from adult mouse tissues. *Nat Genet* **45:**1198-1206; 2013.

[13] Ziller, M. J.; Gu, H.; Muller, F.; Donaghey, J.; Tsai, L. T.; Kohlbacher, O.; De Jager, P. L.; Rosen, E. D.; Bennett, D. A.; Bernstein, B. E.; Gnirke, A.; Meissner, A. Charting a dynamic DNA methylation landscape of the human genome. *Nature* **500:**477-481; 2013.

[14] Bannister, A. J.; Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res* **21:**381-395; 2011.

[15] Garcia, B. A.; Hake, S. B.; Diaz, R. L.; Kauer, M.; Morris, S. A.; Recht, J.; Shabanowitz, J.; Mishra, N.; Strahl, B. D.; Allis, C. D.; Hunt, D. F. Organismal differences in post-translational modifications in histones H3 and H4. *J Biol Chem* **282:**7641-7655; 2007.

[16] Gardner, K. E.; Zhou, L.; Parra, M. A.; Chen, X.; Strahl, B. D. Identification of lysine 37 of histone H2B as a novel site of methylation. *PLoS One* **6:**e16244; 2011.

[17] Hake, S. B.; Garcia, B. A.; Duncan, E. M.; Kauer, M.; Dellaire, G.; Shabanowitz, J.; Bazett-Jones, D. P.; Allis, C. D.; Hunt, D. F. Expression patterns and post-translational modifications associated with mammalian histone H3 variants. *J Biol Chem* **281:**559-568; 2006.

[18] Tan, M.; Luo, H.; Lee, S.; Jin, F.; Yang, J. S.; Montellier, E.; Buchou, T.; Cheng, Z.; Rousseaux, S.; Rajagopal, N.; Lu, Z.; Ye, Z.; Zhu, Q.; Wysocka, J.; Ye, Y.; Khochbin, S.; Ren, B.; Zhao, Y. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* **146:**1016-1028; 2011.

[19] Waldmann, T.; Izzo, A.; Kamieniarz, K.; Richter, F.; Vogler, C.; Sarg, B.; Lindner, H.; Young, N. L.; Mittler, G.; Garcia, B. A.; Schneider, R. Methylation of H2AR29 is a novel repressive PRMT6 target. *Epigenetics Chromatin* **4:**11; 2011.

[20] Kooistra, S. M.; Helin, K. Molecular mechanisms and potential functions of histone demethylases. *Nat Rev Mol Cell Biol* **13:**297-311; 2012.
[21] Jung, H. R.; Pasini, D.; Helin, K.; Jensen, O. N. Quantitative mass spectrometry of histones H3.2 and H3.3 in Suz12-deficient mouse embryonic stem cells reveals distinct, dynamic post-translational modifications at Lys-27 and Lys-36. *Mol Cell Proteomics* **9:**838-850; 2010.
[22] Schotta, G.; Sengupta, R.; Kubicek, S.; Malin, S.; Kauer, M.; Callen, E.; Celeste, A.; Pagani, M.; Opravil, S.; De La Rosa-Velazquez, I. A.; Espejo, A.; Bedford, M. T.; Nussenzweig, A.; Busslinger, M.; Jenuwein, T. A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. *Genes Dev* **22:**2048-2061; 2008.
[23] Haladyna, J. N.; Yamauchi, T.; Neff, T.; Bernt, K. M. Epigenetic modifiers in normal and malignant hematopoiesis. *Epigenomics* **7:**301-320; 2015.
[24] Zhang, G.; Pradhan, S. Mammalian epigenetic mechanisms. *IUBMB Life* **66:**240-256; 2014.
[25] Liu, K.; Wang, Y. F.; Cantemir, C.; Muller, M. T. Endogenous assays of DNA methyltransferases: Evidence for differential activities of DNMT1, DNMT2, and DNMT3 in mammalian cells in vivo. *Mol Cell Biol* **23:**2709-2719; 2003.
[26] Teperino, R.; Schoonjans, K.; Auwerx, J. Histone methyl transferases and demethylases; can they link metabolism and transcription? *Cell Metab* **12:**321-327; 2010.
[27] Greer, E. L.; Shi, Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* **13:**343-357; 2012.
[28] Lu, C.; Thompson, C. B. Metabolic regulation of epigenetics. *Cell Metab* **16:**9-17; 2012.
[29] Islam, M. S.; Leissing, T. M.; Chowdhury, R.; Hopkinson, R. J.; Schofield, C. J. 2-Oxoglutarate-Dependent Oxygenases. *Annu Rev Biochem* **87:**585-620; 2018.
[30] Rose, N. R.; McDonough, M. A.; King, O. N.; Kawamura, A.; Schofield, C. J. Inhibition of 2-oxoglutarate dependent oxygenases. *Chem Soc Rev* **40:**4364-4397; 2011.
[31] Loenarz, C.; Schofield, C. J. Expanding chemical biology of 2-oxoglutarate oxygenases. *Nat Chem Biol* **4:**152-156; 2008.
[32] Martinez, S.; Hausinger, R. P. Catalytic Mechanisms of Fe(II)- and 2-Oxoglutarate-dependent Oxygenases. *J Biol Chem* **290:**20702-20711; 2015.
[33] Tahiliani, M.; Koh, K. P.; Shen, Y.; Pastor, W. A.; Bandukwala, H.; Brudno, Y.; Agarwal, S.; Iyer, L. M.; Liu, D. R.; Aravind, L.; Rao, A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324:**930-935; 2009.
[34] Lorsbach, R. B.; Moore, J.; Mathew, S.; Raimondi, S. C.; Mukatira, S. T.; Downing, J. R. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). *Leukemia* **17:**637-641; 2003.
[35] Ono, R.; Taki, T.; Taketani, T.; Taniwaki, M.; Kobayashi, H.; Hayashi, Y. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11)(q22;q23). *Cancer Res* **62:**4075-4080; 2002.
[36] He, Y. F.; Li, B. Z.; Li, Z.; Liu, P.; Wang, Y.; Tang, Q.; Ding, J.; Jia, Y.; Chen, Z.; Li, L.; Sun, Y.; Li, X.; Dai, Q.; Song, C. X.; Zhang, K.; He, C.; Xu, G. L. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* **333:**1303-1307; 2011.
[37] Ito, S.; Shen, L.; Dai, Q.; Wu, S. C.; Collins, L. B.; Swenberg, J. A.; He, C.; Zhang, Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* **333:**1300-1303; 2011.
[38] Wu, X.; Zhang, Y. TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat Rev Genet* **18:**517-534; 2017.
[39] Bachman, M.; Uribe-Lewis, S.; Yang, X.; Burgess, H. E.; Iurlaro, M.; Reik, W.; Murrell, A.; Balasubramanian, S. 5-Formylcytosine can be a stable DNA modification in mammals. *Nat Chem Biol* **11:**555-557; 2015.
[40] Bachman, M.; Uribe-Lewis, S.; Yang, X.; Williams, M.; Murrell, A.; Balasubramanian, S. 5-Hydroxymethylcytosine is a predominantly stable DNA modification. *Nat Chem* **6:**1049-1055; 2014.

[41] An, J.; Rao, A.; Ko, M. TET family dioxygenases and DNA demethylation in stem cells and cancers. *Exp Mol Med* **49:**e323; 2017.
[42] Franchini, D. M.; Schmitz, K. M.; Petersen-Mahrt, S. K. 5-Methylcytosine DNA demethylation: more than losing a methyl group. *Annu Rev Genet* **46:**419-441; 2012.
[43] Rasmussen, K. D.; Helin, K. Role of TET enzymes in DNA methylation, development, and cancer. *Genes Dev* **30:**733-750; 2016.
[44] Burr, S.; Caldwell, A.; Chong, M.; Beretta, M.; Metcalf, S.; Hancock, M.; Arno, M.; Balu, S.; Kropf, V. L.; Mistry, R. K.; Shah, A. M.; Mann, G. E.; Brewer, A. C. Oxygen gradients can determine epigenetic asymmetry and cellular differentiation via differential regulation of Tet activity in embryonic stem cells. *Nucleic Acids Res* **46:**1210-1226; 2018.
[45] Hu, L.; Lu, J.; Cheng, J.; Rao, Q.; Li, Z.; Hou, H.; Lou, Z.; Zhang, L.; Li, W.; Gong, W.; Liu, M.; Sun, C.; Yin, X.; Li, J.; Tan, X.; Wang, P.; Wang, Y.; Fang, D.; Cui, Q.; Yang, P.; He, C.; Jiang, H.; Luo, C.; Xu, Y. Structural insight into substrate preference for TET-mediated oxidation. *Nature* **527:**118-122; 2015.
[46] Pfaffeneder, T.; Spada, F.; Wagner, M.; Brandmayr, C.; Laube, S. K.; Eisen, D.; Truss, M.; Steinbacher, J.; Hackner, B.; Kotljarova, O.; Schuermann, D.; Michalakis, S.; Kosmatchev, O.; Schiesser, S.; Steigenberger, B.; Raddaoui, N.; Kashiwazaki, G.; Muller, U.; Spruijt, C. G.; Vermeulen, M.; Leonhardt, H.; Schar, P.; Muller, M.; Carell, T. Tet oxidizes thymine to 5-hydroxymethyluracil in mouse embryonic stem cell DNA. *Nat Chem Biol* **10:**574-581; 2014.
[47] Pastor, W. A.; Aravind, L.; Rao, A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. *Nat Rev Mol Cell Biol* **14:**341-356; 2013.
[48] Ito, S.; D'Alessio, A. C.; Taranova, O. V.; Hong, K.; Sowers, L. C.; Zhang, Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* **466:**1129-1133; 2010.
[49] Zhang, H.; Zhang, X.; Clark, E.; Mulcahey, M.; Huang, S.; Shi, Y. G. TET1 is a DNA-binding protein that modulates DNA methylation and gene transcription via hydroxylation of 5-methylcytosine. *Cell Res* **20:**1390-1393; 2010.
[50] Arioka, Y.; Watanabe, A.; Saito, K.; Yamada, Y. Activation-induced cytidine deaminase alters the subcellular localization of Tet family proteins. *PLoS One* **7:**e45031; 2012.
[51] Kohli, R. M.; Zhang, Y. TET enzymes, TDG and the dynamics of DNA demethylation. *Nature* **502:**472-479; 2013.
[52] Ko, M.; An, J.; Bandukwala, H. S.; Chavez, L.; Aijo, T.; Pastor, W. A.; Segal, M. F.; Li, H.; Koh, K. P.; Lahdesmaki, H.; Hogan, P. G.; Aravind, L.; Rao, A. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. *Nature* **497:**122-126; 2013.
[53] Good, C. R.; Madzo, J.; Patel, B.; Maegawa, S.; Engel, N.; Jelinek, J.; Issa, J. J. A novel isoform of TET1 that lacks a CXXC domain is overexpressed in cancer. *Nucleic Acids Res* **45:**8269-8281; 2017.
[54] Jin, S. G.; Zhang, Z. M.; Dunwell, T. L.; Harter, M. R.; Wu, X.; Johnson, J.; Li, Z.; Liu, J.; Szabo, P. E.; Lu, Q.; Xu, G. L.; Song, J.; Pfeifer, G. P. Tet3 Reads 5-Carboxylcytosine through Its CXXC Domain and Is a Potential Guardian against Neurodegeneration. *Cell Rep* **14:**493-505; 2016.
[55] Mohr, F.; Dohner, K.; Buske, C.; Rawat, V. P. TET genes: new players in DNA demethylation and important determinants for stemness. *Exp Hematol* **39:**272-281; 2011.
[56] Zheng, Y. C.; Ma, J.; Wang, Z.; Li, J.; Jiang, B.; Zhou, W.; Shi, X.; Wang, X.; Zhao, W.; Liu, H. M. A Systematic Review of Histone Lysine-Specific Demethylase 1 and Its Inhibitors. *Med Res Rev* **35:**1032-1071; 2015.
[57] Takeuchi, T.; Yamazaki, Y.; Katoh-Fukui, Y.; Tsuchiya, R.; Kondo, S.; Motoyama, J.; Higashinakagawa, T. Gene trap capture of a novel mouse gene, jumonji, required for neural tube formation. *Genes Dev* **9:**1211-1222; 1995.
[58] Shmakova, A.; Batie, M.; Druker, J.; Rocha, S. Chromatin and oxygen sensing in the context of JmjC histone demethylases. *Biochem J* **462:**385-395; 2014.

[59] Jia, B.; Tang, K.; Chun, B. H.; Jeon, C. O. Large-scale examination of functional and sequence diversity of 2-oxoglutarate/Fe(II)-dependent oxygenases in Metazoa. *Biochim Biophys Acta* **1861:**2922-2933; 2017.
[60] Johansson, C.; Tumber, A.; Che, K.; Cain, P.; Nowak, R.; Gileadi, C.; Oppermann, U. The roles of Jumonji-type oxygenases in human disease. *Epigenomics* **6:**89-120; 2014.
[61] Pedersen, M. T.; Helin, K. Histone demethylases in development and disease. *Trends Cell Biol* **20:**662-671; 2010.
[62] Schofield, C. J.; Ratcliffe, P. J. Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* **5:**343-354; 2004.
[63] Lancaster, D. E.; McDonough, M. A.; Schofield, C. J. Factor inhibiting hypoxia-inducible factor (FIH) and other asparaginyl hydroxylases. *Biochem Soc Trans* **32:**943-945; 2004.
[64] Nakazawa, M. S.; Keith, B.; Simon, M. C. Oxygen availability and metabolic adaptations. *Nat Rev Cancer* **16:**663-673; 2016.
[65] Koh, K. P.; Yabuuchi, A.; Rao, S.; Huang, Y.; Cunniff, K.; Nardone, J.; Laiho, A.; Tahiliani, M.; Sommer, C. A.; Mostoslavsky, G.; Lahesmaa, R.; Orkin, S. H.; Rodig, S. J.; Daley, G. Q.; Rao, A. Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. *Cell Stem Cell* **8:**200-213; 2011.
[66] Li, X.; Yue, X.; Pastor, W. A.; Lin, L.; Georges, R.; Chavez, L.; Evans, S. M.; Rao, A. Tet proteins influence the balance between neuroectodermal and mesodermal fate choice by inhibiting Wnt signaling. *Proc Natl Acad Sci U S A* **113:**E8267-E8276; 2016.
[67] Huang, Y.; Rao, A. Connections between TET proteins and aberrant DNA modification in cancer. *Trends Genet* **30:**464-474; 2014.
[68] Laukka, T.; Mariani, C. J.; Ihantola, T.; Cao, J. Z.; Hokkanen, J.; Kaelin, W. G., Jr.; Godley, L. A.; Koivunen, P. Fumarate and Succinate Regulate Expression of Hypoxia-inducible Genes via TET Enzymes. *J Biol Chem* **291:**4256-4265; 2016.
[69] Hirsila, M.; Koivunen, P.; Gunzler, V.; Kivirikko, K. I.; Myllyharju, J. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. *J Biol Chem* **278:**30772-30780; 2003.
[70] Koivunen, P.; Hirsila, M.; Gunzler, V.; Kivirikko, K. I.; Myllyharju, J. Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. *J Biol Chem* **279:**9899-9904; 2004.
[71] Thienpont, B.; Steinbacher, J.; Zhao, H.; D'Anna, F.; Kuchnio, A.; Ploumakis, A.; Ghesquiere, B.; Van Dyck, L.; Boeckx, B.; Schoonjans, L.; Hermans, E.; Amant, F.; Kristensen, V. N.; Peng Koh, K.; Mazzone, M.; Coleman, M.; Carell, T.; Carmeliet, P.; Lambrechts, D. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. *Nature* **537:**63-68; 2016.
[72] Jeschke, J.; Collignon, E.; Fuks, F. Portraits of TET-mediated DNA hydroxymethylation in cancer. *Curr Opin Genet Dev* **36:**16-26; 2016.
[73] Keeley, T. P.; Mann, G. E. Defining Physiological Normoxia for Improved Translation of Cell Physiology to Animal Models and Humans. *Physiol Rev* **99:**161-234; 2019.
[74] Place, T. L.; Domann, F. E.; Case, A. J. Limitations of oxygen delivery to cells in culture: An underappreciated problem in basic and translational research. *Free Radic Biol Med* **113:**311-322; 2017.
[75] Buffa, F. M.; Harris, A. L.; West, C. M.; Miller, C. J. Large meta-analysis of multiple cancers reveals a common, compact and highly prognostic hypoxia metagene. *Br J Cancer* **102:**428-435; 2010.
[76] Dawlaty, M. M.; Breiling, A.; Le, T.; Raddatz, G.; Barrasa, M. I.; Cheng, A. W.; Gao, Q.; Powell, B. E.; Li, Z.; Xu, M.; Faull, K. F.; Lyko, F.; Jaenisch, R. Combined deficiency of Tet1 and Tet2 causes epigenetic abnormalities but is compatible with postnatal development. *Dev Cell* **24:**310-323; 2013.
[77] Dawlaty, M. M.; Ganz, K.; Powell, B. E.; Hu, Y. C.; Markoulaki, S.; Cheng, A. W.; Gao, Q.; Kim, J.; Choi, S. W.; Page, D. C.; Jaenisch, R. Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. *Cell Stem Cell* **9:**166-175; 2011.

[78] Freudenberg, J. M.; Ghosh, S.; Lackford, B. L.; Yellaboina, S.; Zheng, X.; Li, R.; Cuddapah, S.; Wade, P. A.; Hu, G.; Jothi, R. Acute depletion of Tet1-dependent 5-hydroxymethylcytosine levels impairs LIF/Stat3 signaling and results in loss of embryonic stem cell identity. *Nucleic Acids Res* **40:**3364-3377; 2012.
[79] Huang, Y.; Chavez, L.; Chang, X.; Wang, X.; Pastor, W. A.; Kang, J.; Zepeda-Martinez, J. A.; Pape, U. J.; Jacobsen, S. E.; Peters, B.; Rao, A. Distinct roles of the methylcytosine oxidases Tet1 and Tet2 in mouse embryonic stem cells. *Proc Natl Acad Sci U S A* **111:**1361-1366; 2014.
[80] Kang, J.; Lienhard, M.; Pastor, W. A.; Chawla, A.; Novotny, M.; Tsagaratou, A.; Lasken, R. S.; Thompson, E. C.; Surani, M. A.; Koralov, S. B.; Kalantry, S.; Chavez, L.; Rao, A. Simultaneous deletion of the methylcytosine oxidases Tet1 and Tet3 increases transcriptome variability in early embryogenesis. *Proc Natl Acad Sci U S A* **112:**E4236-4245; 2015.
[81] Khoueiry, R.; Sohni, A.; Thienpont, B.; Luo, X.; Velde, J. V.; Bartoccetti, M.; Boeckx, B.; Zwijsen, A.; Rao, A.; Lambrechts, D.; Koh, K. P. Lineage-specific functions of TET1 in the postimplantation mouse embryo. *Nat Genet* **49:**1061-1072; 2017.
[82] Li, C.; Lan, Y.; Schwartz-Orbach, L.; Korol, E.; Tahiliani, M.; Evans, T.; Goll, M. G. Overlapping Requirements for Tet2 and Tet3 in Normal Development and Hematopoietic Stem Cell Emergence. *Cell Rep* **12:**1133-1143; 2015.
[83] Rudenko, A.; Dawlaty, M. M.; Seo, J.; Cheng, A. W.; Meng, J.; Le, T.; Faull, K. F.; Jaenisch, R.; Tsai, L. H. Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. *Neuron* **79:**1109-1122; 2013.
[84] Wu, H.; D'Alessio, A. C.; Ito, S.; Xia, K.; Wang, Z.; Cui, K.; Zhao, K.; Sun, Y. E.; Zhang, Y. Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. *Nature* **473:**389-393; 2011.
[85] Zhang, R. R.; Cui, Q. Y.; Murai, K.; Lim, Y. C.; Smith, Z. D.; Jin, S.; Ye, P.; Rosa, L.; Lee, Y. K.; Wu, H. P.; Liu, W.; Xu, Z. M.; Yang, L.; Ding, Y. Q.; Tang, F.; Meissner, A.; Ding, C.; Shi, Y.; Xu, G. L. Tet1 regulates adult hippocampal neurogenesis and cognition. *Cell Stem Cell* **13:**237-245; 2013.
[86] Maltepe, E.; Simon, M. C. Oxygen, genes, and development: an analysis of the role of hypoxic gene regulation during murine vascular development. *J Mol Med (Berl)* **76:**391-401; 1998.
[87] Mohyeldin, A.; Garzon-Muvdi, T.; Quinones-Hinojosa, A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* **7:**150-161; 2010.
[88] Simon, M. C.; Keith, B. The role of oxygen availability in embryonic development and stem cell function. *Nat Rev Mol Cell Biol* **9:**285-296; 2008.
[89] Takahashi, M. Oxidative stress and redox regulation on in vitro development of mammalian embryos. *J Reprod Dev* **58:**1-9; 2012.
[90] Alivand, M. R.; Soheili, Z. S.; Pornour, M.; Solali, S.; Sabouni, F. Novel Epigenetic Controlling of Hypoxia Pathway Related to Overexpression and Promoter Hypomethylation of TET1 and TET2 in RPE Cells. *J Cell Biochem* **118:**3193-3204; 2017.
[91] Chen, H. F.; Wu, K. J. Epigenetics, TET proteins, and hypoxia in epithelial-mesenchymal transition and tumorigenesis. *Biomedicine (Taipei)* **6:**1; 2016.
[92] Kao, S. H.; Wu, K. J.; Lee, W. H. Hypoxia, Epithelial-Mesenchymal Transition, and TET-Mediated Epigenetic Changes. *J Clin Med* **5**; 2016.
[93] Lin, G.; Sun, W.; Yang, Z.; Guo, J.; Liu, H.; Liang, J. Hypoxia induces the expression of TET enzymes in HepG2 cells. *Oncol Lett* **14:**6457-6462; 2017.
[94] Prasad, P.; Mittal, S. A.; Chongtham, J.; Mohanty, S.; Srivastava, T. Hypoxia-Mediated Epigenetic Regulation of Stemness in Brain Tumor Cells. *Stem Cells* **35:**1468-1478; 2017.
[95] Mariani, C. J.; Vasanthakumar, A.; Madzo, J.; Yesilkanal, A.; Bhagat, T.; Yu, Y.; Bhattacharyya, S.; Wenger, R. H.; Cohn, S. L.; Nanduri, J.; Verma, A.; Prabhakar, N. R.; Godley, L. A. TET1-mediated hydroxymethylation facilitates hypoxic gene induction in neuroblastoma. *Cell Rep* **7:**1343-1352; 2014.
[96] Wu, M. Z.; Chen, S. F.; Nieh, S.; Benner, C.; Ger, L. P.; Jan, C. I.; Ma, L.; Chen, C. H.; Hishida, T.; Chang, H. T.; Lin, Y. S.; Montserrat, N.; Gascon, P.; Sancho-Martinez, I.; Izpisua Belmonte, J. C.

Hypoxia Drives Breast Tumor Malignancy through a TET-TNFalpha-p38-MAPK Signaling Axis. *Cancer Res* **75:**3912-3924; 2015.

[97] Li, T.; Yang, D.; Li, J.; Tang, Y.; Yang, J.; Le, W. Critical role of Tet3 in neural progenitor cell maintenance and terminal differentiation. *Mol Neurobiol* **51:**142-154; 2015.

[98] Xu, Y.; Xu, C.; Kato, A.; Tempel, W.; Abreu, J. G.; Bian, C.; Hu, Y.; Hu, D.; Zhao, B.; Cerovina, T.; Diao, J.; Wu, F.; He, H. H.; Cui, Q.; Clark, E.; Ma, C.; Barbara, A.; Veenstra, G. J.; Xu, G.; Kaiser, U. B.; Liu, X. S.; Sugrue, S. P.; He, X.; Min, J.; Kato, Y.; Shi, Y. G. Tet3 CXXC domain and dioxygenase activity cooperatively regulate key genes for Xenopus eye and neural development. *Cell* **151:**1200-1213; 2012.

[99] Varier, R. A.; Timmers, H. T. Histone lysine methylation and demethylation pathways in cancer. *Biochim Biophys Acta* **1815:**75-89; 2011.

[100] Hancock, R. L.; Dunne, K.; Walport, L. J.; Flashman, E.; Kawamura, A. Epigenetic regulation by histone demethylases in hypoxia. *Epigenomics* **7:**791-811; 2015.

[101] Johnson, A. B.; Denko, N.; Barton, M. C. Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. *Mutat Res* **640:**174-179; 2008.

[102] Tausendschon, M.; Dehne, N.; Brune, B. Hypoxia causes epigenetic gene regulation in macrophages by attenuating Jumonji histone demethylase activity. *Cytokine* **53:**256-262; 2011.

[103] Zhou, X.; Sun, H.; Chen, H.; Zavadil, J.; Kluz, T.; Arita, A.; Costa, M. Hypoxia induces trimethylated H3 lysine 4 by inhibition of JARID1A demethylase. *Cancer Res* **70:**4214-4221; 2010.

[104] Sanchez-Fernandez, E. M.; Tarhonskaya, H.; Al-Qahtani, K.; Hopkinson, R. J.; McCullagh, J. S.; Schofield, C. J.; Flashman, E. Investigations on the oxygen dependence of a 2-oxoglutarate histone demethylase. *Biochem J* **449:**491-496; 2013.

[105] Hancock, R. L.; Masson, N.; Dunne, K.; Flashman, E.; Kawamura, A. The Activity of JmjC Histone Lysine Demethylase KDM4A is Highly Sensitive to Oxygen Concentrations. *ACS Chem Biol* **12:**1011-1019; 2017.

[106] Beyer, S.; Kristensen, M. M.; Jensen, K. S.; Johansen, J. V.; Staller, P. The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. *J Biol Chem* **283:**36542-36552; 2008.

[107] Voigt, P.; Tee, W. W.; Reinberg, D. A double take on bivalent promoters. *Genes Dev* **27:**1318-1338; 2013.

[108] Intlekofer, A. M.; Dematteo, R. G.; Venneti, S.; Finley, L. W.; Lu, C.; Judkins, A. R.; Rustenburg, A. S.; Grinaway, P. B.; Chodera, J. D.; Cross, J. R.; Thompson, C. B. Hypoxia Induces Production of L-2-Hydroxyglutarate. *Cell Metab* **22:**304-311; 2015.

[109] Niu, X.; Zhang, T.; Liao, L.; Zhou, L.; Lindner, D. J.; Zhou, M.; Rini, B.; Yan, Q.; Yang, H. The von Hippel-Lindau tumor suppressor protein regulates gene expression and tumor growth through histone demethylase JARID1C. *Oncogene* **31:**776-786; 2012.

[110] Pollard, P. J.; Loenarz, C.; Mole, D. R.; McDonough, M. A.; Gleadle, J. M.; Schofield, C. J.; Ratcliffe, P. J. Regulation of Jumonji-domain-containing histone demethylases by hypoxia-inducible factor (HIF)-1alpha. *Biochem J* **416:**387-394; 2008.

[111] Wellmann, S.; Bettkober, M.; Zelmer, A.; Seeger, K.; Faigle, M.; Eltzschig, H. K.; Buhrer, C. Hypoxia upregulates the histone demethylase JMJD1A via HIF-1. *Biochem Biophys Res Commun* **372:**892-897; 2008.

[112] Yang, J.; Jubb, A. M.; Pike, L.; Buffa, F. M.; Turley, H.; Baban, D.; Leek, R.; Gatter, K. C.; Ragoussis, J.; Harris, A. L. The histone demethylase JMJD2B is regulated by estrogen receptor alpha and hypoxia, and is a key mediator of estrogen induced growth. *Cancer Res* **70:**6456-6466; 2010.

[113] Yang, J.; Ledaki, I.; Turley, H.; Gatter, K. C.; Montero, J. C.; Li, J. L.; Harris, A. L. Role of hypoxia-inducible factors in epigenetic regulation via histone demethylases. *Ann N Y Acad Sci* **1177:**185-197; 2009.

[114] Melvin, A.; Rocha, S. Chromatin as an oxygen sensor and active player in the hypoxia response. *Cell Signal* **24:**35-43; 2012.

[115] Xia, X.; Lemieux, M. E.; Li, W.; Carroll, J. S.; Brown, M.; Liu, X. S.; Kung, A. L. Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis. *Proc Natl Acad Sci U S A* **106:**4260-4265; 2009.
[116] Krieg, A. J.; Rankin, E. B.; Chan, D.; Razorenova, O.; Fernandez, S.; Giaccia, A. J. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. *Mol Cell Biol* **30:**344-353; 2010.
[117] Luo, W.; Chang, R.; Zhong, J.; Pandey, A.; Semenza, G. L. Histone demethylase JMJD2C is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. *Proc Natl Acad Sci U S A* **109:**E3367-3376; 2012.
[118] Loenarz, C.; Schofield, C. J. Physiological and biochemical aspects of hydroxylations and demethylations catalyzed by human 2-oxoglutarate oxygenases. *Trends Biochem Sci* **36:**7-18; 2011.
[119] Ozer, A.; Bruick, R. K. Non-heme dioxygenases: cellular sensors and regulators jelly rolled into one? *Nat Chem Biol* **3:**144-153; 2007.
[120] Mantri, M.; Zhang, Z.; McDonough, M. A.; Schofield, C. J. Autocatalysed oxidative modifications to 2-oxoglutarate dependent oxygenases. *FEBS J* **279:**1563-1575; 2012.
[121] Hoffart, L. M.; Barr, E. W.; Guyer, R. B.; Bollinger, J. M., Jr.; Krebs, C. Direct spectroscopic detection of a C-H-cleaving high-spin Fe(IV) complex in a prolyl-4-hydroxylase. *Proc Natl Acad Sci U S A* **103:**14738-14743; 2006.
[122] Kuiper, C.; Vissers, M. C. Ascorbate as a co-factor for fe- and 2-oxoglutarate dependent dioxygenases: physiological activity in tumor growth and progression. *Front Oncol* **4:**359; 2014.
[123] Vissers, M. C.; Gunningham, S. P.; Morrison, M. J.; Dachs, G. U.; Currie, M. J. Modulation of hypoxia-inducible factor-1 alpha in cultured primary cells by intracellular ascorbate. *Free Radic Biol Med* **42:**765-772; 2007.
[124] Cash, T. P.; Pan, Y.; Simon, M. C. Reactive oxygen species and cellular oxygen sensing. *Free Radic Biol Med* **43:**1219-1225; 2007.
[125] Chandel, N. S.; McClintock, D. S.; Feliciano, C. E.; Wood, T. M.; Melendez, J. A.; Rodriguez, A. M.; Schumacker, P. T. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. *J Biol Chem* **275:**25130-25138; 2000.
[126] Guzy, R. D.; Schumacker, P. T. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp Physiol* **91:**807-819; 2006.
[127] Guzy, R. D.; Sharma, B.; Bell, E.; Chandel, N. S.; Schumacker, P. T. Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol* **28:**718-731; 2008.
[128] Haddad, J. J.; Land, S. C. A non-hypoxic, ROS-sensitive pathway mediates TNF-alpha-dependent regulation of HIF-1alpha. *FEBS Lett* **505:**269-274; 2001.
[129] Li, Y. N.; Xi, M. M.; Guo, Y.; Hai, C. X.; Yang, W. L.; Qin, X. J. NADPH oxidase-mitochondria axis-derived ROS mediate arsenite-induced HIF-1alpha stabilization by inhibiting prolyl hydroxylases activity. *Toxicol Lett* **224:**165-174; 2014.
[130] Mansfield, K. D.; Guzy, R. D.; Pan, Y.; Young, R. M.; Cash, T. P.; Schumacker, P. T.; Simon, M. C. Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. *Cell Metab* **1:**393-399; 2005.
[131] Brunelle, J. K.; Bell, E. L.; Quesada, N. M.; Vercauteren, K.; Tiranti, V.; Zeviani, M.; Scarpulla, R. C.; Chandel, N. S. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. *Cell Metab* **1:**409-414; 2005.
[132] Gerald, D.; Berra, E.; Frapart, Y. M.; Chan, D. A.; Giaccia, A. J.; Mansuy, D.; Pouyssegur, J.; Yaniv, M.; Mechta-Grigoriou, F. JunD reduces tumor angiogenesis by protecting cells from oxidative stress. *Cell* **118:**781-794; 2004.
[133] Lopez-Lazaro, M. Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy. *Cancer Lett* **252:**1-8; 2007.

[134] Herr, B.; Zhou, J.; Drose, S.; Brune, B. The interaction of superoxide with nitric oxide destabilizes hypoxia-inducible factor-1alpha. *Cell Mol Life Sci* **64:**3295-3305; 2007.
[135] Kozhukhar, A. V.; Yasinska, I. M.; Sumbayev, V. V. Nitric oxide inhibits HIF-1alpha protein accumulation under hypoxic conditions: implication of 2-oxoglutarate and iron. *Biochimie* **88:**411-418; 2006.
[136] Sumbayev, V. V.; Yasinska, I. M. Peroxynitrite as an alternative donor of oxygen in HIF-1alpha proline hydroxylation under low oxygen availability. *Free Radic Res* **40:**631-635; 2006.
[137] Yang, Z. Z.; Zhang, A. Y.; Yi, F. X.; Li, P. L.; Zou, A. P. Redox regulation of HIF-1alpha levels and HO-1 expression in renal medullary interstitial cells. *Am J Physiol Renal Physiol* **284:**F1207-1215; 2003.
[138] Al Taleb, Z.; Petry, A.; Chi, T. F.; Mennerich, D.; Gorlach, A.; Dimova, E. Y.; Kietzmann, T. Differential transcriptional regulation of hypoxia-inducible factor-1alpha by arsenite under normoxia and hypoxia: involvement of Nrf2. *J Mol Med (Berl)* **94:**1153-1166; 2016.
[139] Bonello, S.; Zahringer, C.; BelAiba, R. S.; Djordjevic, T.; Hess, J.; Michiels, C.; Kietzmann, T.; Gorlach, A. Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. *Arterioscler Thromb Vasc Biol* **27:**755-761; 2007.
[140] Lacher, S. E.; Levings, D. C.; Freeman, S.; Slattery, M. Identification of a functional antioxidant response element at the HIF1A locus. *Redox Biol* **19:**401-411; 2018.
[141] Gorlach, A.; Dimova, E. Y.; Petry, A.; Martinez-Ruiz, A.; Hernansanz-Agustin, P.; Rolo, A. P.; Palmeira, C. M.; Kietzmann, T. Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? *Redox Biol* **6:**372-385; 2015.
[142] Gorlach, A.; Kietzmann, T. Superoxide and derived reactive oxygen species in the regulation of hypoxia-inducible factors. *Methods Enzymol* **435:**421-446; 2007.
[143] Schieber, M.; Chandel, N. S. ROS function in redox signaling and oxidative stress. *Curr Biol* **24:**R453-462; 2014.
[144] Chiarugi, P. PTPs versus PTKs: the redox side of the coin. *Free Radic Res* **39:**353-364; 2005.
[145] Lee, G.; Won, H. S.; Lee, Y. M.; Choi, J. W.; Oh, T. I.; Jang, J. H.; Choi, D. K.; Lim, B. O.; Kim, Y. J.; Park, J. W.; Puigserver, P.; Lim, J. H. Oxidative Dimerization of PHD2 is Responsible for its Inactivation and Contributes to Metabolic Reprogramming via HIF-1alpha Activation. *Sci Rep* **6:**18928; 2016.
[146] Emerling, B. M.; Platanias, L. C.; Black, E.; Nebreda, A. R.; Davis, R. J.; Chandel, N. S. Mitochondrial reactive oxygen species activation of p38 mitogen-activated protein kinase is required for hypoxia signaling. *Mol Cell Biol* **25:**4853-4862; 2005.
[147] Pan, Y.; Mansfield, K. D.; Bertozzi, C. C.; Rudenko, V.; Chan, D. A.; Giaccia, A. J.; Simon, M. C. Multiple factors affecting cellular redox status and energy metabolism modulate hypoxia-inducible factor prolyl hydroxylase activity in vivo and in vitro. *Mol Cell Biol* **27:**912-925; 2007.
[148] Minor, E. A.; Court, B. L.; Young, J. I.; Wang, G. Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine. *J Biol Chem* **288:**13669-13674; 2013.
[149] Niu, Y.; DesMarais, T. L.; Tong, Z.; Yao, Y.; Costa, M. Oxidative stress alters global histone modification and DNA methylation. *Free Radic Biol Med* **82:**22-28; 2015.
[150] Munzel, T.; Daiber, A. Environmental Stressors and Their Impact on Health and Disease with Focus on Oxidative Stress. *Antioxid Redox Signal* **28:**735-740; 2018.
[151] Coulter, J. B.; O'Driscoll, C. M.; Bressler, J. P. Hydroquinone increases 5-hydroxymethylcytosine formation through ten eleven translocation 1 (TET1) 5-methylcytosine dioxygenase. *J Biol Chem* **288:**28792-28800; 2013.
[152] Zhao, B.; Yang, Y.; Wang, X.; Chong, Z.; Yin, R.; Song, S. H.; Zhao, C.; Li, C.; Huang, H.; Sun, B. F.; Wu, D.; Jin, K. X.; Song, M.; Zhu, B. Z.; Jiang, G.; Rendtlew Danielsen, J. M.; Xu, G. L.; Yang, Y. G.; Wang, H. Redox-active quinones induces genome-wide DNA methylation changes by an iron-mediated and Tet-dependent mechanism. *Nucleic Acids Res* **42:**1593-1605; 2014.

[153] Galaris, D.; Skiada, V.; Barbouti, A. Redox signaling and cancer: the role of "labile" iron. *Cancer Lett* **266:**21-29; 2008.
[154] Shao, D.; Oka, S.; Liu, T.; Zhai, P.; Ago, T.; Sciarretta, S.; Li, H.; Sadoshima, J. A redox-dependent mechanism for regulation of AMPK activation by Thioredoxin1 during energy starvation. *Cell Metab* **19:**232-245; 2014.
[155] Wu, D.; Hu, D.; Chen, H.; Shi, G.; Fetahu, I. S.; Wu, F.; Rabidou, K.; Fang, R.; Tan, L.; Xu, S.; Liu, H.; Argueta, C.; Zhang, L.; Mao, F.; Yan, G.; Chen, J.; Dong, Z.; Lv, R.; Xu, Y.; Wang, M.; Ye, Y.; Zhang, S.; Duquette, D.; Geng, S.; Yin, C.; Lian, C. G.; Murphy, G. F.; Adler, G. K.; Garg, R.; Lynch, L.; Yang, P.; Li, Y.; Lan, F.; Fan, J.; Shi, Y.; Shi, Y. G. Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. *Nature* **559:**637-641; 2018.
[156] Seisenberger, S.; Andrews, S.; Krueger, F.; Arand, J.; Walter, J.; Santos, F.; Popp, C.; Thienpont, B.; Dean, W.; Reik, W. The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol Cell* **48:**849-862; 2012.
[157] Smith, Z. D.; Chan, M. M.; Mikkelsen, T. S.; Gu, H.; Gnirke, A.; Regev, A.; Meissner, A. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484:**339-344; 2012.
[158] Blaschke, K.; Ebata, K. T.; Karimi, M. M.; Zepeda-Martinez, J. A.; Goyal, P.; Mahapatra, S.; Tam, A.; Laird, D. J.; Hirst, M.; Rao, A.; Lorincz, M. C.; Ramalho-Santos, M. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. *Nature* **500:**222-226; 2013.
[159] Chen, J.; Guo, L.; Zhang, L.; Wu, H.; Yang, J.; Liu, H.; Wang, X.; Hu, X.; Gu, T.; Zhou, Z.; Liu, J.; Liu, J.; Wu, H.; Mao, S. Q.; Mo, K.; Li, Y.; Lai, K.; Qi, J.; Yao, H.; Pan, G.; Xu, G. L.; Pei, D. Vitamin C modulates TET1 function during somatic cell reprogramming. *Nat Genet* **45:**1504-1509; 2013.
[160] Yu, X. X.; Liu, Y. H.; Liu, X. M.; Wang, P. C.; Liu, S.; Miao, J. K.; Du, Z. Q.; Yang, C. X. Ascorbic acid induces global epigenetic reprogramming to promote meiotic maturation and developmental competence of porcine oocytes. *Sci Rep* **8:**6132; 2018.
[161] Qu, K.; Ma, X. F.; Li, G. H.; Zhang, H.; Liu, Y. M.; Zhang, K.; Zeng, J. F.; Lei, J. J.; Wei, D. H.; Wang, Z. Vitamin C down-regulate apo(a) expression via Tet2-dependent DNA demethylation in HepG2 cells. *Int J Biol Macromol* **98:**637-645; 2017.
[162] Lai, C. H.; Liou, S. H.; Lin, H. C.; Shih, T. S.; Tsai, P. J.; Chen, J. S.; Yang, T.; Jaakkola, J. J.; Strickland, P. T. Exposure to traffic exhausts and oxidative DNA damage. *Occup Environ Med* **62:**216-222; 2005.
[163] Somineni, H. K.; Zhang, X.; Biagini Myers, J. M.; Kovacic, M. B.; Ulm, A.; Jurcak, N.; Ryan, P. H.; Khurana Hershey, G. K.; Ji, H. Ten-eleven translocation 1 (TET1) methylation is associated with childhood asthma and traffic-related air pollution. *J Allergy Clin Immunol* **137:**797-805 e795; 2016.
[164] Kang, K. A.; Piao, M. J.; Kim, K. C.; Kang, H. K.; Chang, W. Y.; Park, I. C.; Keum, Y. S.; Surh, Y. J.; Hyun, J. W. Epigenetic modification of Nrf2 in 5-fluorouracil-resistant colon cancer cells: involvement of TET-dependent DNA demethylation. *Cell Death Dis* **5:**e1183; 2014.
[165] Mikhed, Y.; Gorlach, A.; Knaus, U. G.; Daiber, A. Redox regulation of genome stability by effects on gene expression, epigenetic pathways and DNA damage/repair. *Redox Biol* **5:**275-289; 2015.
[166] Chen, H.; Ke, Q.; Kluz, T.; Yan, Y.; Costa, M. Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing. *Mol Cell Biol* **26:**3728-3737; 2006.
[167] Zhou, X.; Li, Q.; Arita, A.; Sun, H.; Costa, M. Effects of nickel, chromate, and arsenite on histone 3 lysine methylation. *Toxicol Appl Pharmacol* **236:**78-84; 2009.
[168] Chervona, Y.; Costa, M. The control of histone methylation and gene expression by oxidative stress, hypoxia, and metals. *Free Radic Biol Med* **53:**1041-1047; 2012.
[169] Jensen, T. J.; Wozniak, R. J.; Eblin, K. E.; Wnek, S. M.; Gandolfi, A. J.; Futscher, B. W. Epigenetic mediated transcriptional activation of WNT5A participates in arsenical-associated malignant transformation. *Toxicol Appl Pharmacol* **235:**39-46; 2009.
[170] Sun, H.; Zhou, X.; Chen, H.; Li, Q.; Costa, M. Modulation of histone methylation and MLH1 gene silencing by hexavalent chromium. *Toxicol Appl Pharmacol* **237:**258-266; 2009.

[171] Zhou, X.; Sun, H.; Ellen, T. P.; Chen, H.; Costa, M. Arsenite alters global histone H3 methylation. *Carcinogenesis* **29:**1831-1836; 2008.
[172] Ricq, E. L.; Hooker, J. M.; Haggarty, S. J. Activity-dependent Regulation of Histone Lysine Demethylase KDM1A by a Putative Thiol/Disulfide Switch. *J Biol Chem* **291:**24756-24767; 2016.
[173] Cyr, A. R.; Domann, F. E. The redox basis of epigenetic modifications: from mechanisms to functional consequences. *Antioxid Redox Signal* **15:**551-589; 2011.
[174] Wang, T.; Chen, K.; Zeng, X.; Yang, J.; Wu, Y.; Shi, X.; Qin, B.; Zeng, L.; Esteban, M. A.; Pan, G.; Pei, D. The histone demethylases Jhdm1a/1b enhance somatic cell reprogramming in a vitamin-C-dependent manner. *Cell Stem Cell* **9:**575-587; 2011.
[175] Motorin, Y.; Helm, M. RNA nucleotide methylation. *Wiley Interdiscip Rev RNA* **2:**611-631; 2011.
[176] Fu, Y.; Dominissini, D.; Rechavi, G.; He, C. Gene expression regulation mediated through reversible m(6)A RNA methylation. *Nat Rev Genet* **15:**293-306; 2014.
[177] Fu, Y.; Jia, G.; Pang, X.; Wang, R. N.; Wang, X.; Li, C. J.; Smemo, S.; Dai, Q.; Bailey, K. A.; Nobrega, M. A.; Han, K. L.; Cui, Q.; He, C. FTO-mediated formation of N6-hydroxymethyladenosine and N6-formyladenosine in mammalian RNA. *Nat Commun* **4:**1798; 2013.
[178] Jia, G.; Fu, Y.; Zhao, X.; Dai, Q.; Zheng, G.; Yang, Y.; Yi, C.; Lindahl, T.; Pan, T.; Yang, Y. G.; He, C. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol* **7:**885-887; 2011.
[179] Fischer, J.; Koch, L.; Emmerling, C.; Vierkotten, J.; Peters, T.; Bruning, J. C.; Ruther, U. Inactivation of the Fto gene protects from obesity. *Nature* **458:**894-898; 2009.
[180] Gerken, T.; Girard, C. A.; Tung, Y. C.; Webby, C. J.; Saudek, V.; Hewitson, K. S.; Yeo, G. S.; McDonough, M. A.; Cunliffe, S.; McNeill, L. A.; Galvanovskis, J.; Rorsman, P.; Robins, P.; Prieur, X.; Coll, A. P.; Ma, M.; Jovanovic, Z.; Farooqi, I. S.; Sedgwick, B.; Barroso, I.; Lindahl, T.; Ponting, C. P.; Ashcroft, F. M.; O'Rahilly, S.; Schofield, C. J. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* **318:**1469-1472; 2007.
[181] Zheng, G.; Dahl, J. A.; Niu, Y.; Fedorcsak, P.; Huang, C. M.; Li, C. J.; Vagbo, C. B.; Shi, Y.; Wang, W. L.; Song, S. H.; Lu, Z.; Bosmans, R. P.; Dai, Q.; Hao, Y. J.; Yang, X.; Zhao, W. M.; Tong, W. M.; Wang, X. J.; Bogdan, F.; Furu, K.; Fu, Y.; Jia, G.; Zhao, X.; Liu, J.; Krokan, H. E.; Klungland, A.; Yang, Y. G.; He, C. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol Cell* **49:**18-29; 2013.
[182] Liu, R.; Jin, Y.; Tang, W. H.; Qin, L.; Zhang, X.; Tellides, G.; Hwa, J.; Yu, J.; Martin, K. A. Ten-eleven translocation-2 (TET2) is a master regulator of smooth muscle cell plasticity. *Circulation* **128:**2047-2057; 2013.
[183] Greco, C. M.; Kunderfranco, P.; Rubino, M.; Larcher, V.; Carullo, P.; Anselmo, A.; Kurz, K.; Carell, T.; Angius, A.; Latronico, M. V.; Papait, R.; Condorelli, G. DNA hydroxymethylation controls cardiomyocyte gene expression in development and hypertrophy. *Nat Commun* **7:**12418; 2016.
[184] Rabkin, S. W.; Klassen, S. S. Jumonji is a potential regulatory factor mediating nitric oxide-induced modulation of cardiac hypertrophy. *J Cardiovasc Med (Hagerstown)* **10:**206-211; 2009.
[185] Polytarchou, C.; Pfau, R.; Hatziapostolou, M.; Tsichlis, P. N. The JmjC domain histone demethylase Ndy1 regulates redox homeostasis and protects cells from oxidative stress. *Mol Cell Biol* **28:**7451-7464; 2008.
[186] Ezkurdia, I.; Juan, D.; Rodriguez, J. M.; Frankish, A.; Diekhans, M.; Harrow, J.; Vazquez, J.; Valencia, A.; Tress, M. L. Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. *Hum Mol Genet* **23:**5866-5878; 2014.
[187] Kim, M. S.; Pinto, S. M.; Getnet, D.; Nirujogi, R. S.; Manda, S. S.; Chaerkady, R.; Madugundu, A. K.; Kelkar, D. S.; Isserlin, R.; Jain, S.; Thomas, J. K.; Muthusamy, B.; Leal-Rojas, P.; Kumar, P.; Sahasrabuddhe, N. A.; Balakrishnan, L.; Advani, J.; George, B.; Renuse, S.; Selvan, L. D.; Patil, A. H.; Nanjappa, V.; Radhakrishnan, A.; Prasad, S.; Subbannayya, T.; Raju, R.; Kumar, M.; Sreenivasamurthy, S. K.; Marimuthu, A.; Sathe, G. J.; Chavan, S.; Datta, K. K.; Subbannayya, Y.; Sahu, A.; Yelamanchi, S. D.; Jayaram, S.; Rajagopalan, P.; Sharma, J.; Murthy, K. R.; Syed, N.; Goel, R.; Khan, A. A.; Ahmad, S.;

Dey, G.; Mudgal, K.; Chatterjee, A.; Huang, T. C.; Zhong, J.; Wu, X.; Shaw, P. G.; Freed, D.; Zahari, M. S.; Mukherjee, K. K.; Shankar, S.; Mahadevan, A.; Lam, H.; Mitchell, C. J.; Shankar, S. K.; Satishchandra, P.; Schroeder, J. T.; Sirdeshmukh, R.; Maitra, A.; Leach, S. D.; Drake, C. G.; Halushka, M. K.; Prasad, T. S.; Hruban, R. H.; Kerr, C. L.; Bader, G. D.; Iacobuzio-Donahue, C. A.; Gowda, H.; Pandey, A. A draft map of the human proteome. *Nature* **509:**575-581; 2014.

[188] Vella, P.; Scelfo, A.; Jammula, S.; Chiacchiera, F.; Williams, K.; Cuomo, A.; Roberto, A.; Christensen, J.; Bonaldi, T.; Helin, K.; Pasini, D. Tet proteins connect the O-linked N-acetylglucosamine transferase Ogt to chromatin in embryonic stem cells. *Mol Cell* **49:**645-656; 2013.

[189] Chrysanthou, S.; Senner, C. E.; Woods, L.; Fineberg, E.; Okkenhaug, H.; Burge, S.; Perez-Garcia, V.; Hemberger, M. A Critical Role of TET1/2 Proteins in Cell-Cycle Progression of Trophoblast Stem Cells. *Stem Cell Reports* **10:**1355-1368; 2018.

[190] Zhu, F.; Zhu, Q.; Ye, D.; Zhang, Q.; Yang, Y.; Guo, X.; Liu, Z.; Jiapaer, Z.; Wan, X.; Wang, G.; Chen, W.; Zhu, S.; Jiang, C.; Shi, W.; Kang, J. Sin3a-Tet1 interaction activates gene transcription and is required for embryonic stem cell pluripotency. *Nucleic Acids Res* **46:**6026-6040; 2018.

[191] Lv, Q. Y.; Xie, B. Y.; Yang, B. Y.; Ning, C. C.; Shan, W. W.; Gu, C.; Luo, X. Z.; Chen, X. J.; Zhang, Z. B.; Feng, Y. J. Increased TET1 Expression in Inflammatory Microenvironment of Hyperinsulinemia Enhances the Response of Endometrial Cancer to Estrogen by Epigenetic Modulation of GPER. *J Cancer* **8:**894-902; 2017.

[192] Huang, H.; Jiang, X.; Li, Z.; Li, Y.; Song, C. X.; He, C.; Sun, M.; Chen, P.; Gurbuxani, S.; Wang, J.; Hong, G. M.; Elkahloun, A. G.; Arnovitz, S.; Wang, J.; Szulwach, K.; Lin, L.; Street, C.; Wunderlich, M.; Dawlaty, M.; Neilly, M. B.; Jaenisch, R.; Yang, F. C.; Mulloy, J. C.; Jin, P.; Liu, P. P.; Rowley, J. D.; Xu, M.; He, C.; Chen, J. TET1 plays an essential oncogenic role in MLL-rearranged leukemia. *Proc Natl Acad Sci U S A* **110:**11994-11999; 2013.

[193] Kudo, Y.; Tateishi, K.; Yamamoto, K.; Yamamoto, S.; Asaoka, Y.; Ijichi, H.; Nagae, G.; Yoshida, H.; Aburatani, H.; Koike, K. Loss of 5-hydroxymethylcytosine is accompanied with malignant cellular transformation. *Cancer Sci* **103:**670-676; 2012.

[194] Lian, C. G.; Xu, Y.; Ceol, C.; Wu, F.; Larson, A.; Dresser, K.; Xu, W.; Tan, L.; Hu, Y.; Zhan, Q.; Lee, C. W.; Hu, D.; Lian, B. Q.; Kleffel, S.; Yang, Y.; Neiswender, J.; Khorasani, A. J.; Fang, R.; Lezcano, C.; Duncan, L. M.; Scolyer, R. A.; Thompson, J. F.; Kakavand, H.; Houvras, Y.; Zon, L. I.; Mihm, M. C., Jr.; Kaiser, U. B.; Schatton, T.; Woda, B. A.; Murphy, G. F.; Shi, Y. G. Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell* **150:**1135-1146; 2012.

[195] Muller, T.; Gessi, M.; Waha, A.; Isselstein, L. J.; Luxen, D.; Freihoff, D.; Freihoff, J.; Becker, A.; Simon, M.; Hammes, J.; Denkhaus, D.; zur Muhlen, A.; Pietsch, T.; Waha, A. Nuclear exclusion of TET1 is associated with loss of 5-hydroxymethylcytosine in IDH1 wild-type gliomas. *Am J Pathol* **181:**675-683; 2012.

[196] Yang, H.; Liu, Y.; Bai, F.; Zhang, J. Y.; Ma, S. H.; Liu, J.; Xu, Z. D.; Zhu, H. G.; Ling, Z. Q.; Ye, D.; Guan, K. L.; Xiong, Y. Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation. *Oncogene* **32:**663-669; 2013.

[197] Moran-Crusio, K.; Reavie, L.; Shih, A.; Abdel-Wahab, O.; Ndiaye-Lobry, D.; Lobry, C.; Figueroa, M. E.; Vasanthakumar, A.; Patel, J.; Zhao, X.; Perna, F.; Pandey, S.; Madzo, J.; Song, C.; Dai, Q.; He, C.; Ibrahim, S.; Beran, M.; Zavadil, J.; Nimer, S. D.; Melnick, A.; Godley, L. A.; Aifantis, I.; Levine, R. L. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **20:**11-24; 2011.

[198] Song, S. J.; Ito, K.; Ala, U.; Kats, L.; Webster, K.; Sun, S. M.; Jongen-Lavrencic, M.; Manova-Todorova, K.; Teruya-Feldstein, J.; Avigan, D. E.; Delwel, R.; Pandolfi, P. P. The oncogenic microRNA miR-22 targets the TET2 tumor suppressor to promote hematopoietic stem cell self-renewal and transformation. *Cell Stem Cell* **13:**87-101; 2013.

[199] Aslanyan, M. G.; Kroeze, L. I.; Langemeijer, S. M.; Koorenhof-Scheele, T. N.; Massop, M.; van Hoogen, P.; Stevens-Linders, E.; van de Locht, L. T.; Tonnissen, E.; van der Heijden, A.; da Silva-Coelho, P.; Cilloni, D.; Saglio, G.; Marie, J. P.; Tang, R.; Labar, B.; Amadori, S.; Muus, P.; Willemze, R.; Marijt, E. W.; de Witte, T.; van der Reijden, B. A.; Suciu, S.; Jansen, J. H. Clinical and biological impact

of TET2 mutations and expression in younger adult AML patients treated within the EORTC/GIMEMA AML-12 clinical trial. *Ann Hematol* **93:**1401-1412; 2014.

[200] Kunimoto, H.; Fukuchi, Y.; Sakurai, M.; Sadahira, K.; Ikeda, Y.; Okamoto, S.; Nakajima, H. Tet2 disruption leads to enhanced self-renewal and altered differentiation of fetal liver hematopoietic stem cells. *Sci Rep* **2:**273; 2012.

[201] Langemeijer, S. M.; Kuiper, R. P.; Berends, M.; Knops, R.; Aslanyan, M. G.; Massop, M.; Stevens-Linders, E.; van Hoogen, P.; van Kessel, A. G.; Raymakers, R. A.; Kamping, E. J.; Verhoef, G. E.; Verburgh, E.; Hagemeijer, A.; Vandenberghe, P.; de Witte, T.; van der Reijden, B. A.; Jansen, J. H. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat Genet* **41:**838-842; 2009.

[202] Viguie, F.; Aboura, A.; Bouscary, D.; Ramond, S.; Delmer, A.; Tachdjian, G.; Marie, J. P.; Casadevall, N. Common 4q24 deletion in four cases of hematopoietic malignancy: early stem cell involvement? *Leukemia* **19:**1411-1415; 2005.

[203] Patnaik, M. M.; Lasho, T. L.; Vijayvargiya, P.; Finke, C. M.; Hanson, C. A.; Ketterling, R. P.; Gangat, N.; Tefferi, A. Prognostic interaction between ASXL1 and TET2 mutations in chronic myelomonocytic leukemia. *Blood Cancer J* **6:**e385; 2016.

[204] Tsukada, Y.; Akiyama, T.; Nakayama, K. I. Maternal TET3 is dispensable for embryonic development but is required for neonatal growth. *Sci Rep* **5:**15876; 2015.

[205] Iqbal, K.; Jin, S. G.; Pfeifer, G. P.; Szabo, P. E. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proc Natl Acad Sci U S A* **108:**3642-3647; 2011.

[206] Guan, W.; Guyot, R.; Samarut, J.; Flamant, F.; Wong, J.; Gauthier, K. C. Methylcytosine dioxygenase TET3 interacts with thyroid hormone nuclear receptors and stabilizes their association to chromatin. *Proc Natl Acad Sci U S A* **114:**8229-8234; 2017.

[207] Xue, S.; Liu, C.; Sun, X.; Li, W.; Zhang, C.; Zhou, X.; Lu, Y.; Xiao, J.; Li, C.; Xu, X.; Sun, B.; Xu, G.; Wang, H. TET3 Inhibits Type I IFN Production Independent of DNA Demethylation. *Cell Rep* **16:**1096-1105; 2016.

[208] An, J.; Gonzalez-Avalos, E.; Chawla, A.; Jeong, M.; Lopez-Moyado, I. F.; Li, W.; Goodell, M. A.; Chavez, L.; Ko, M.; Rao, A. Acute loss of TET function results in aggressive myeloid cancer in mice. *Nat Commun* **6:**10071; 2015.

[209] Lu, T.; Jackson, M. W.; Wang, B.; Yang, M.; Chance, M. R.; Miyagi, M.; Gudkov, A. V.; Stark, G. R. Regulation of NF-kappaB by NSD1/FBXL11-dependent reversible lysine methylation of p65. *Proc Natl Acad Sci U S A* **107:**46-51; 2010.

[210] Borgel, J.; Tyl, M.; Schiller, K.; Pusztai, Z.; Dooley, C. M.; Deng, W.; Wooding, C.; White, R. J.; Warnecke, T.; Leonhardt, H.; Busch-Nentwich, E. M.; Bartke, T. KDM2A integrates DNA and histone modification signals through a CXXC/PHD module and direct interaction with HP1. *Nucleic Acids Res* **45:**1114-1129; 2017.

[211] Chen, J. Y.; Luo, C. W.; Lai, Y. S.; Wu, C. C.; Hung, W. C. Lysine demethylase KDM2A inhibits TET2 to promote DNA methylation and silencing of tumor suppressor genes in breast cancer. *Oncogenesis* **6:**e369; 2017.

[212] Zhou, Z.; Yang, X.; He, J.; Liu, J.; Wu, F.; Yu, S.; Liu, Y.; Lin, R.; Liu, H.; Cui, Y.; Zhou, C.; Wang, X.; Wu, J.; Cao, S.; Guo, L.; Lin, L.; Wang, T.; Peng, X.; Qiang, B.; Hutchins, A. P.; Pei, D.; Chen, J. Kdm2b Regulates Somatic Reprogramming through Variant PRC1 Complex-Dependent Function. *Cell Rep* **21:**2160-2170; 2017.

[213] Ge, R.; Wang, Z.; Zeng, Q.; Xu, X.; Olumi, A. F. F-box protein 10, an NF-kappaB-dependent anti-apoptotic protein, regulates TRAIL-induced apoptosis through modulating c-Fos/c-FLIP pathway. *Cell Death Differ* **18:**1184-1195; 2011.

[214] Tzatsos, A.; Paskaleva, P.; Ferrari, F.; Deshpande, V.; Stoykova, S.; Contino, G.; Wong, K. K.; Lan, F.; Trojer, P.; Park, P. J.; Bardeesy, N. KDM2B promotes pancreatic cancer via Polycomb-dependent and -independent transcriptional programs. *J Clin Invest* **123:**727-739; 2013.

[215] Zheng, Q.; Fan, H.; Meng, Z.; Yuan, L.; Liu, C.; Peng, Y.; Zhao, W.; Wang, L.; Li, J.; Feng, J. Histone demethylase KDM2B promotes triple negative breast cancer proliferation by suppressing p15INK4B, p16INK4A, and p57KIP2 transcription. *Acta Biochim Biophys Sin (Shanghai)*; 2018.
[216] Cho, H. S.; Toyokawa, G.; Daigo, Y.; Hayami, S.; Masuda, K.; Ikawa, N.; Yamane, Y.; Maejima, K.; Tsunoda, T.; Field, H. I.; Kelly, J. D.; Neal, D. E.; Ponder, B. A.; Maehara, Y.; Nakamura, Y.; Hamamoto, R. The JmjC domain-containing histone demethylase KDM3A is a positive regulator of the G1/S transition in cancer cells via transcriptional regulation of the HOXA1 gene. *Int J Cancer* **131:**E179-189; 2012.
[217] Wilson, S.; Fan, L.; Sahgal, N.; Qi, J.; Filipp, F. V. The histone demethylase KDM3A regulates the transcriptional program of the androgen receptor in prostate cancer cells. *Oncotarget* **8:**30328-30343; 2017.
[218] Ramadoss, S.; Sen, S.; Ramachandran, I.; Roy, S.; Chaudhuri, G.; Farias-Eisner, R. Lysine-specific demethylase KDM3A regulates ovarian cancer stemness and chemoresistance. *Oncogene* **36:**1537-1545; 2017.
[219] Xu, X.; Nagel, S.; Quentmeier, H.; Wang, Z.; Pommerenke, C.; Dirks, W. G.; Macleod, R. A. F.; Drexler, H. G.; Hu, Z. KDM3B shows tumor-suppressive activity and transcriptionally regulates HOXA1 through retinoic acid response elements in acute myeloid leukemia. *Leuk Lymphoma* **59:**204-213; 2018.
[220] Liu, Z.; Chen, X.; Zhou, S.; Liao, L.; Jiang, R.; Xu, J. The histone H3K9 demethylase Kdm3b is required for somatic growth and female reproductive function. *Int J Biol Sci* **11:**494-507; 2015.
[221] Li, J.; Yu, B.; Deng, P.; Cheng, Y.; Yu, Y.; Kevork, K.; Ramadoss, S.; Ding, X.; Li, X.; Wang, C. Y. KDM3 epigenetically controls tumorigenic potentials of human colorectal cancer stem cells through Wnt/beta-catenin signalling. *Nat Commun* **8:**15146; 2017.
[222] Kim, J. Y.; Kim, K. B.; Eom, G. H.; Choe, N.; Kee, H. J.; Son, H. J.; Oh, S. T.; Kim, D. W.; Pak, J. H.; Baek, H. J.; Kook, H.; Hahn, Y.; Kook, H.; Chakravarti, D.; Seo, S. B. KDM3B is the H3K9 demethylase involved in transcriptional activation of lmo2 in leukemia. *Mol Cell Biol* **32:**2917-2933; 2012.
[223] Dobrynin, G.; McAllister, T. E.; Leszczynska, K. B.; Ramachandran, S.; Krieg, A. J.; Kawamura, A.; Hammond, E. M. KDM4A regulates HIF-1 levels through H3K9me3. *Sci Rep* **7:**11094; 2017.
[224] Guerra-Calderas, L.; Gonzalez-Barrios, R.; Herrera, L. A.; Cantu de Leon, D.; Soto-Reyes, E. The role of the histone demethylase KDM4A in cancer. *Cancer Genet* **208:**215-224; 2015.
[225] Wang, L. Y.; Hung, C. L.; Chen, Y. R.; Yang, J. C.; Wang, J.; Campbell, M.; Izumiya, Y.; Chen, H. W.; Wang, W. C.; Ann, D. K.; Kung, H. J. KDM4A Coactivates E2F1 to Regulate the PDK-Dependent Metabolic Switch between Mitochondrial Oxidation and Glycolysis. *Cell Rep* **16:**3016-3027; 2016.
[226] Young, L. C.; McDonald, D. W.; Hendzel, M. J. Kdm4b histone demethylase is a DNA damage response protein and confers a survival advantage following gamma-irradiation. *J Biol Chem* **288:**21376-21388; 2013.
[227] Gaughan, L.; Stockley, J.; Coffey, K.; O'Neill, D.; Jones, D. L.; Wade, M.; Wright, J.; Moore, M.; Tse, S.; Rogerson, L.; Robson, C. N. KDM4B is a master regulator of the estrogen receptor signalling cascade. *Nucleic Acids Res* **41:**6892-6904; 2013.
[228] Wilson, C.; Qiu, L.; Hong, Y.; Karnik, T.; Tadros, G.; Mau, B.; Ma, T.; Mu, Y.; New, J.; Louie, R. J.; Gunewardena, S.; Godwin, A. K.; Tawfik, O. W.; Chien, J.; Roby, K. F.; Krieg, A. J. The histone demethylase KDM4B regulates peritoneal seeding of ovarian cancer. *Oncogene* **36:**2565-2576; 2017.
[229] Garcia, J.; Lizcano, F. KDM4C Activity Modulates Cell Proliferation and Chromosome Segregation in Triple-Negative Breast Cancer. *Breast Cancer (Auckl)* **10:**169-175; 2016.
[230] Yamamoto, S.; Tateishi, K.; Kudo, Y.; Yamamoto, K.; Isagawa, T.; Nagae, G.; Nakatsuka, T.; Asaoka, Y.; Ijichi, H.; Hirata, Y.; Otsuka, M.; Ikenoue, T.; Aburatani, H.; Omata, M.; Koike, K. Histone demethylase KDM4C regulates sphere formation by mediating the cross talk between Wnt and Notch pathways in colonic cancer cells. *Carcinogenesis* **34:**2380-2388; 2013.

[231] Wu, R.; Wang, Z.; Zhang, H.; Gan, H.; Zhang, Z. H3K9me3 demethylase Kdm4d facilitates the formation of pre-initiative complex and regulates DNA replication. *Nucleic Acids Res* **45:**169-180; 2017.
[232] Khoury-Haddad, H.; Nadar-Ponniah, P. T.; Awwad, S.; Ayoub, N. The emerging role of lysine demethylases in DNA damage response: dissecting the recruitment mode of KDM4D/JMJD2D to DNA damage sites. *Cell Cycle* **14:**950-958; 2015.
[233] Isohookana, J.; Haapasaari, K. M.; Soini, Y.; Karihtala, P. KDM4D Predicts Recurrence in Exocrine Pancreatic Cells of Resection Margins from Patients with Pancreatic Adenocarcinoma. *Anticancer Res* **38:**2295-2302; 2018.
[234] Blair, L. P.; Liu, Z.; Labitigan, R. L.; Wu, L.; Zheng, D.; Xia, Z.; Pearson, E. L.; Nazeer, F. I.; Cao, J.; Lang, S. M.; Rines, R. J.; Mackintosh, S. G.; Moore, C. L.; Li, W.; Tian, B.; Tackett, A. J.; Yan, Q. KDM5 lysine demethylases are involved in maintenance of 3'UTR length. *Sci Adv* **2:**e1501662; 2016.
[235] Feng, T.; Wang, Y.; Lang, Y.; Zhang, Y. KDM5A promotes proliferation and EMT in ovarian cancer and closely correlates with PTX resistance. *Mol Med Rep* **16:**3573-3580; 2017.
[236] Wang, X.; Zhou, M.; Fu, Y.; Sun, T.; Chen, J.; Qin, X.; Yu, Y.; Jia, J.; Chen, C. RBP2 Promotes Adult Acute Lymphoblastic Leukemia by Upregulating BCL2. *PLoS One* **11:**e0152142; 2016.
[237] Wang, Z. Y.; Yang, J.; Liu, C. K.; Shen, S. Q. High Expression of Retinoblastoma-Binding Protein 2 (RBP2) in Patients with Hepatocellular Carcinoma and Its Prognostic Significance. *Med Sci Monit* **23:**2736-2744; 2017.
[238] Zhou, D.; Kannappan, V.; Chen, X.; Li, J.; Leng, X.; Zhang, J.; Xuan, S. RBP2 induces stem-like cancer cells by promoting EMT and is a prognostic marker for renal cell carcinoma. *Exp Mol Med* **48:**e238; 2016.
[239] Kuzbicki, L.; Lange, D.; Stanek-Widera, A.; Chwirot, B. W. Prognostic significance of RBP2-H1 variant of JARID1B in melanoma. *BMC Cancer* **17:**854; 2017.
[240] Fang, L.; Zhao, J.; Wang, D.; Zhu, L.; Wang, J.; Jiang, K. Jumonji AT-rich interactive domain 1B overexpression is associated with the development and progression of glioma. *Int J Mol Med* **38:**172-182; 2016.
[241] Lin, C. S.; Lin, Y. C.; Adebayo, B. O.; Wu, A.; Chen, J. H.; Peng, Y. J.; Cheng, M. F.; Lee, W. H.; Hsiao, M.; Chao, T. Y.; Yeh, C. T. Silencing JARID1B suppresses oncogenicity, stemness and increases radiation sensitivity in human oral carcinoma. *Cancer Lett* **368:**36-45; 2015.
[242] Xu, L.; Wu, W.; Cheng, G.; Qian, M.; Hu, K.; Yin, G.; Wang, S. Enhancement of Proliferation and Invasion of Gastric Cancer Cell by KDM5C Via Decrease in p53 Expression. *Technol Cancer Res Treat* **16:**141-149; 2017.
[243] Denis, H.; Van Grembergen, O.; Delatte, B.; Dedeurwaerder, S.; Putmans, P.; Calonne, E.; Rothe, F.; Sotiriou, C.; Fuks, F.; Deplus, R. MicroRNAs regulate KDM5 histone demethylases in breast cancer cells. *Mol Biosyst* **12:**404-413; 2016.
[244] Wang, W.; Meadows, L. R.; den Haan, J. M.; Sherman, N. E.; Chen, Y.; Blokland, E.; Shabanowitz, J.; Agulnik, A. I.; Hendrickson, R. C.; Bishop, C. E.; et al. Human H-Y: a male-specific histocompatibility antigen derived from the SMCY protein. *Science* **269:**1588-1590; 1995.
[245] Li, N.; Dhar, S. S.; Chen, T. Y.; Kan, P. Y.; Wei, Y.; Kim, J. H.; Chan, C. H.; Lin, H. K.; Hung, M. C.; Lee, M. G. JARID1D Is a Suppressor and Prognostic Marker of Prostate Cancer Invasion and Metastasis. *Cancer Res* **76:**831-843; 2016.
[246] Jiang, W.; Wang, J.; Zhang, Y. Histone H3K27me3 demethylases KDM6A and KDM6B modulate definitive endoderm differentiation from human ESCs by regulating WNT signaling pathway. *Cell Res* **23:**122-130; 2013.
[247] Hurst, C. D.; Alder, O.; Platt, F. M.; Droop, A.; Stead, L. F.; Burns, J. E.; Burghel, G. J.; Jain, S.; Klimczak, L. J.; Lindsay, H.; Roulson, J. A.; Taylor, C. F.; Thygesen, H.; Cameron, A. J.; Ridley, A. J.; Mott, H. R.; Gordenin, D. A.; Knowles, M. A. Genomic Subtypes of Non-invasive Bladder Cancer with Distinct Metabolic Profile and Female Gender Bias in KDM6A Mutation Frequency. *Cancer Cell* **32:**701-715 e707; 2017.

[248] Banka, S.; Lederer, D.; Benoit, V.; Jenkins, E.; Howard, E.; Bunstone, S.; Kerr, B.; McKee, S.; Lloyd, I. C.; Shears, D.; Stewart, H.; White, S. M.; Savarirayan, R.; Mancini, G. M.; Beysen, D.; Cohn, R. D.; Grisart, B.; Maystadt, I.; Donnai, D. Novel KDM6A (UTX) mutations and a clinical and molecular review of the X-linked Kabuki syndrome (KS2). *Clin Genet* **87:**252-258; 2015.
[249] Zhang, Y.; Shen, L.; Stupack, D. G.; Bai, N.; Xun, J.; Ren, G.; Han, J.; Li, L.; Luo, Y.; Xiang, R.; Tan, X. JMJD3 promotes survival of diffuse large B-cell lymphoma subtypes via distinct mechanisms. *Oncotarget* **7:**29387-29399; 2016.
[250] Ohguchi, H.; Harada, T.; Sagawa, M.; Kikuchi, S.; Tai, Y. T.; Richardson, P. G.; Hideshima, T.; Anderson, K. C. KDM6B modulates MAPK pathway mediating multiple myeloma cell growth and survival. *Leukemia* **31:**2661-2669; 2017.
[251] Huang, C.; Xiang, Y.; Wang, Y.; Li, X.; Xu, L.; Zhu, Z.; Zhang, T.; Zhu, Q.; Zhang, K.; Jing, N.; Chen, C. D. Dual-specificity histone demethylase KIAA1718 (KDM7A) regulates neural differentiation through FGF4. *Cell Res* **20:**154-165; 2010.
[252] Choi, J. Y.; Jo, S. A. KDM7A histone demethylase mediates TNF-alpha-induced ICAM1 protein upregulation by modulating lysosomal activity. *Biochem Biophys Res Commun* **478:**1355-1362; 2016.
[253] Osawa, T.; Muramatsu, M.; Wang, F.; Tsuchida, R.; Kodama, T.; Minami, T.; Shibuya, M. Increased expression of histone demethylase JHDM1D under nutrient starvation suppresses tumor growth via down-regulating angiogenesis. *Proc Natl Acad Sci U S A* **108:**20725-20729; 2011.
[254] Shi, G.; Wu, M.; Fang, L.; Yu, F.; Cheng, S.; Li, J.; Du, J. X.; Wong, J. PHD finger protein 2 (PHF2) represses ribosomal RNA gene transcription by antagonizing PHF finger protein 8 (PHF8) and recruiting methyltransferase SUV39H1. *J Biol Chem* **289:**29691-29700; 2014.
[255] Ulicna, L.; Kalendova, A.; Kalasova, I.; Vacik, T.; Hozak, P. PIP2 epigenetically represses rRNA genes transcription interacting with PHF8. *Biochim Biophys Acta* **1863:**266-275; 2018.
[256] Fu, Y.; Yang, Y.; Wang, X.; Yin, X.; Zhou, M.; Wang, S.; Yang, L.; Huang, T.; Xu, M.; Chen, C. The histone demethylase PHF8 promotes adult acute lymphoblastic leukemia through interaction with the MEK/ERK signaling pathway. *Biochem Biophys Res Commun* **496:**981-987; 2018.
[257] Maina, P. K.; Shao, P.; Liu, Q.; Fazli, L.; Tyler, S.; Nasir, M.; Dong, X.; Qi, H. H. c-MYC drives histone demethylase PHF8 during neuroendocrine differentiation and in castration-resistant prostate cancer. *Oncotarget* **7:**75585-75602; 2016.
[258] Shao, P.; Liu, Q.; Maina, P. K.; Cui, J.; Bair, T. B.; Li, T.; Umesalma, S.; Zhang, W.; Qi, H. H. Histone demethylase PHF8 promotes epithelial to mesenchymal transition and breast tumorigenesis. *Nucleic Acids Res* **45:**1687-1702; 2017.
[259] Shen, Y.; Pan, X.; Zhao, H. The histone demethylase PHF8 is an oncogenic protein in human non-small cell lung cancer. *Biochem Biophys Res Commun* **451:**119-125; 2014.
[260] Lee, J. H.; Yoo, N. J.; Kim, M. S.; Lee, S. H. Histone Demethylase Gene PHF2 Is Mutated in Gastric and Colorectal Cancers. *Pathol Oncol Res* **23:**471-476; 2017.
[261] Ge, Z.; Gu, Y.; Han, Q.; Sloane, J.; Ge, Q.; Gao, G.; Ma, J.; Song, H.; Hu, J.; Chen, B.; Dovat, S.; Song, C. Plant homeodomain finger protein 2 as a novel IKAROS target in acute lymphoblastic leukemia. *Epigenomics* **10:**59-69; 2018.
[262] Lee, K. H.; Park, J. W.; Sung, H. S.; Choi, Y. J.; Kim, W. H.; Lee, H. S.; Chung, H. J.; Shin, H. W.; Cho, C. H.; Kim, T. Y.; Li, S. H.; Youn, H. D.; Kim, S. J.; Chun, Y. S. PHF2 histone demethylase acts as a tumor suppressor in association with p53 in cancer. *Oncogene* **34:**2897-2909; 2015.
[263] Huang, X.; Zhang, S.; Qi, H.; Wang, Z.; Chen, H. W.; Shao, J.; Shen, J. JMJD5 interacts with p53 and negatively regulates p53 function in control of cell cycle and proliferation. *Biochim Biophys Acta* **1853:**2286-2295; 2015.
[264] Zhang, R.; Huang, Q.; Li, Y.; Song, Y.; Li, Y. JMJD5 is a potential oncogene for colon carcinogenesis. *Int J Clin Exp Pathol* **8:**6482-6489; 2015.
[265] Wang, W.; Lu, Y.; Stemmer, P. M.; Zhang, X.; Bi, Y.; Yi, Z.; Chen, F. The proteomic investigation reveals interaction of mdig protein with the machinery of DNA double-strand break repair. *Oncotarget* **6:**28269-28281; 2015.

[266] Yu, M.; Sun, J.; Thakur, C.; Chen, B.; Lu, Y.; Zhao, H.; Chen, F. Paradoxical roles of mineral dust induced gene on cell proliferation and migration/invasion. *PLoS One* **9:**e87998; 2014.
[267] Tan, X. P.; Dong, W. G.; Zhang, Q.; Yang, Z. R.; Lei, X. F.; Ai, M. H. Potential effects of Mina53 on tumor growth in human pancreatic cancer. *Cell Biochem Biophys* **69:**619-625; 2014.
[268] Geng, F.; Jiang, Z.; Song, X.; Zhou, H.; Zhao, H. Mdig suppresses epithelial-mesenchymal transition and inhibits the invasion and metastasis of nonsmall cell lung cancer via regulating GSK-3beta/beta-catenin signaling. *Int J Oncol* **51:**1898-1908; 2017.
[269] Huang, M. Y.; Xuan, F.; Liu, W.; Cui, H. J. MINA controls proliferation and tumorigenesis of glioblastoma by epigenetically regulating cyclins and CDKs via H3K9me3 demethylation. *Oncogene* **36:**387-396; 2017.
[270] Eilbracht, J.; Reichenzeller, M.; Hergt, M.; Schnolzer, M.; Heid, H.; Stohr, M.; Franke, W. W.; Schmidt-Zachmann, M. S. NO66, a highly conserved dual location protein in the nucleolus and in a special type of synchronously replicating chromatin. *Mol Biol Cell* **15:**1816-1832; 2004.
[271] Tao, Y.; Wu, M.; Zhou, X.; Yin, W.; Hu, B.; de Crombrugghe, B.; Sinha, K. M.; Zang, J. Structural insights into histone demethylase NO66 in interaction with osteoblast-specific transcription factor osterix and gene repression. *J Biol Chem* **288:**16430-16437; 2013.
[272] Nishizawa, Y.; Nishida, N.; Konno, M.; Kawamoto, K.; Asai, A.; Koseki, J.; Takahashi, H.; Haraguchi, N.; Nishimura, J.; Hata, T.; Matsuda, C.; Mizushima, T.; Satoh, T.; Doki, Y.; Mori, M.; Ishii, H. Clinical Significance of Histone Demethylase NO66 in Invasive Colorectal Cancer. *Ann Surg Oncol* **24:**841-849; 2017.
[273] Pires-Luis, A. S.; Vieira-Coimbra, M.; Vieira, F. Q.; Costa-Pinheiro, P.; Silva-Santos, R.; Dias, P. C.; Antunes, L.; Lobo, F.; Oliveira, J.; Goncalves, C. S.; Costa, B. M.; Henrique, R.; Jeronimo, C. Expression of histone methyltransferases as novel biomarkers for renal cell tumor diagnosis and prognostication. *Epigenetics* **10:**1033-1043; 2015.
[274] Koivunen, P.; Hirsila, M.; Remes, A. M.; Hassinen, I. E.; Kivirikko, K. I.; Myllyharju, J. Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. *J Biol Chem* **282:**4524-4532; 2007.
[275] Dao, J. H.; Kurzeja, R. J.; Morachis, J. M.; Veith, H.; Lewis, J.; Yu, V.; Tegley, C. M.; Tagari, P. Kinetic characterization and identification of a novel inhibitor of hypoxia-inducible factor prolyl hydroxylase 2 using a time-resolved fluorescence resonance energy transfer-based assay technology. *Anal Biochem* **384:**213-223; 2009.
[276] Ehrismann, D.; Flashman, E.; Genn, D. N.; Mathioudakis, N.; Hewitson, K. S.; Ratcliffe, P. J.; Schofield, C. J. Studies on the activity of the hypoxia-inducible-factor hydroxylases using an oxygen consumption assay. *Biochem J* **401:**227-234; 2007.
[277] Tarhonskaya, H.; Chowdhury, R.; Leung, I. K.; Loik, N. D.; McCullagh, J. S.; Claridge, T. D.; Schofield, C. J.; Flashman, E. Investigating the contribution of the active site environment to the slow reaction of hypoxia-inducible factor prolyl hydroxylase domain 2 with oxygen. *Biochem J* **463:**363-372; 2014.
[278] Tarhonskaya, H.; Hardy, A. P.; Howe, E. A.; Loik, N. D.; Kramer, H. B.; McCullagh, J. S.; Schofield, C. J.; Flashman, E. Kinetic Investigations of the Role of Factor Inhibiting Hypoxia-inducible Factor (FIH) as an Oxygen Sensor. *J Biol Chem* **290:**19726-19742; 2015.
[279] Castella, B. Kinetic Analysis and Inhibition Studies of Iron-Dependent Histone Demethylases. *Arts & Sciences Electronic Theses and Dissertations*; 2014.
[280] Koivunen, P.; Laukka, T. The TET enzymes. *Cell Mol Life Sci* **75:**1339-1348; 2018.
[281] Wigle, T. J.; Swinger, K. K.; Campbell, J. E.; Scholle, M. D.; Sherrill, J.; Admirand, E. A.; Boriack-Sjodin, P. A.; Kuntz, K. W.; Chesworth, R.; Moyer, M. P.; Scott, M. P.; Copeland, R. A. A High-Throughput Mass Spectrometry Assay Coupled with Redox Activity Testing Reduces Artifacts and False Positives in Lysine Demethylase Screening. *J Biomol Screen* **20:**810-820; 2015.

Legends to figures

Figure 1. DNA and histone demethylation pathways. A. Removal of all modified bases can be passive, *via* DNA replication. Active demethylation involves the successive oxidative conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5 hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) by TET proteins. These marks can subsequently be lost upon replication, or can be converted to unmodified cytosine *via* the action of Thymine DNA Glycosylase (TDG) and base excision repair (BER) pathway. SAM; S-adenosyl methionine, DNMT; DNA methyltransferase. B. JmjC-mediated hydroxylation of mono-, di- and tri-methyl-lysine. The oxidative decarboxylation of α-ketoglutarate forms CO_2, succinate, and a reactive iron (IV)- oxo intermediate which then leads to further lysine hydroxylation to yield hemiaminal. This hemiaminal due to its unstable nature is further fragmented to release formaldehyde and non-methylated lysine residue.

Figure 2.

Schematic depicting the chromosomal locations, gene structures and best-characterised protein isoforms of the human TET proteins. All contain the C-terminal catalytic domain comprising the double-stranded β-helix domain (DSBH) with Fe(II) and 2-OG binding sites and an adjacent Cys-rich region. Additionally, TET1 and TET3 have a DNA binding, CXXC domain made up of two Cys4-type zinc finger motifs at their N-termini. The start of translation is indicated (arrowed ATG). Coding exon sequences are shaded.

Figure 3.

Schematic depicting how the differential activities of the TET proteins (which we have observed in HEK 293 cells, over a gradient of 0.5 to 5% O_2) might combine, in the early embryo to generate specific regions of low (back slashed box), intermediate (forward slashed box) or high (speckled box) levels of 5hmC. These differential levels of 5hmC might then act to facilitate spatial-specific patterns of gene expression, leading to distinct differentiation programmes. TET1 activity increases asymptotically, TET 2 activity remains unchanged and TET3 activity exhibits a bell-shaped curve over this graded range of oxygen.

Figure 4.

Schematic of the association of available oxygen concentration and TET function. A reduction in the O_2 substrate can result in reduced TET catalytic activity. In opposition to this, the expression of TETs can be induced under more hypoxic conditions by HIF. Increased expression of TETs can then result in increased 5hmC which can interact with, and promote the binding of HIF to HIF-regulated promoters. Finally, hypoxia-induced, increased expression of TET1/TET3 has been shown to result in the direct interaction with the TNFα promoter to induce expression and downstream p38-MAPK signalling. CS; catalytic site.

Figure 5.

Suggested mechanisms by which altered redox state or the production of pro-oxidants might reduce the activity of 2-oxoglutarate dioxygenases. Oxidation of the ferrous iron due to the intracellular production of H_2O_2, or due to lowered levels of (reduced) ascorbate resulting from cellular oxidative stress could inactivate the catalytic site (CS) of the enzyme. Cysteine thiol (SH) oxidation by H_2O_2 could result in protein dimerization and inactivation. H_2O_2-mediated inactivation of a protein

tyrosine phosphatase (PTP) resulting in activation of a protein tyrosine kinase (PTK) and post-translational modification (phosphorylation (P) of a tyrosine (Tyr) residue) could inactivate the enzyme. Finally, the concentrations of the obligatory 2-oxoglutarate (2-OG) might increase, and levels of the inhibitory metabolites; succinate (Suc) and fumarate (Fum), might decrease, as a result of free radical production associated with mitochondrial dysfunction. GSH; reduced glutathione, GSSG; oxidised glutathione.

Figure 1A

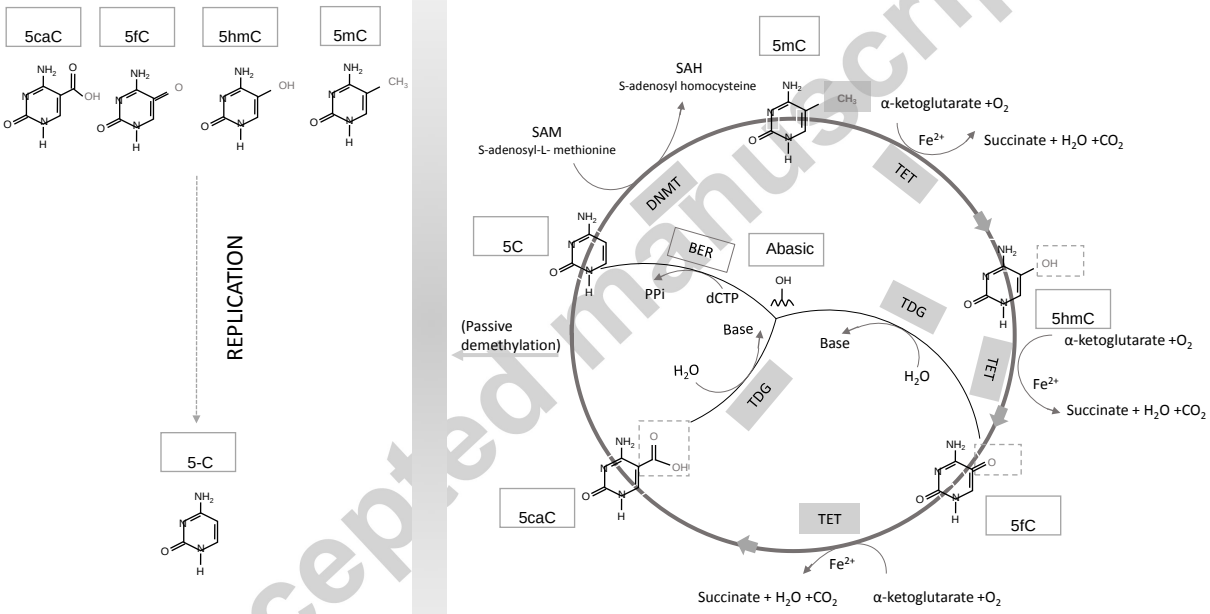


Figure 2

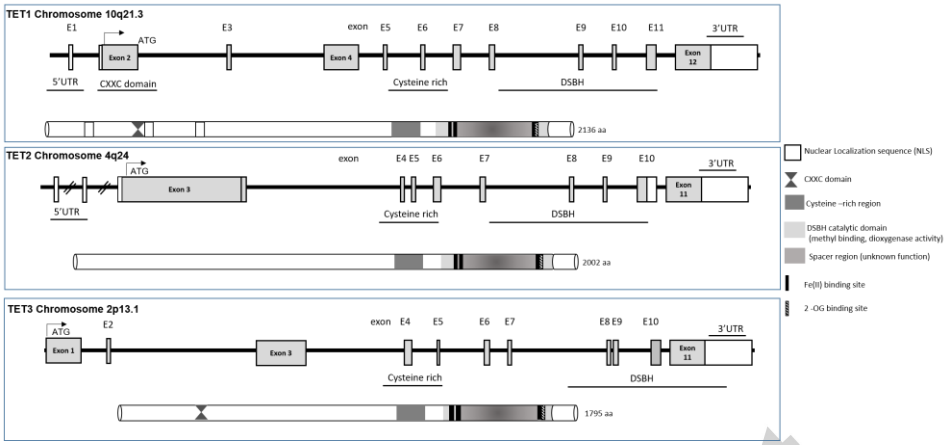


Figure 3

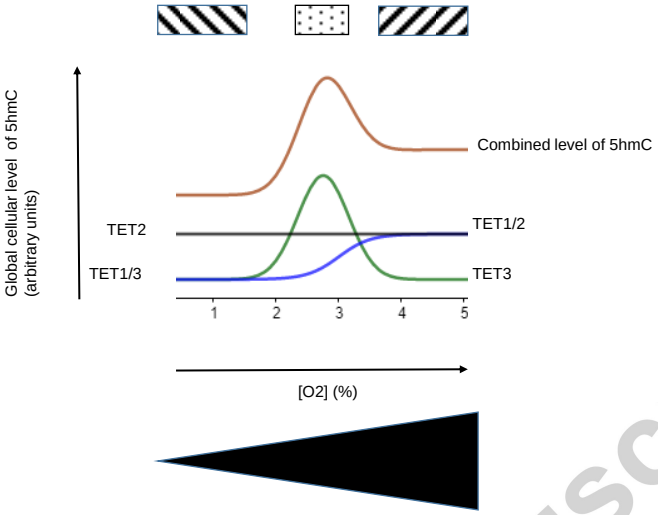


Figure 4

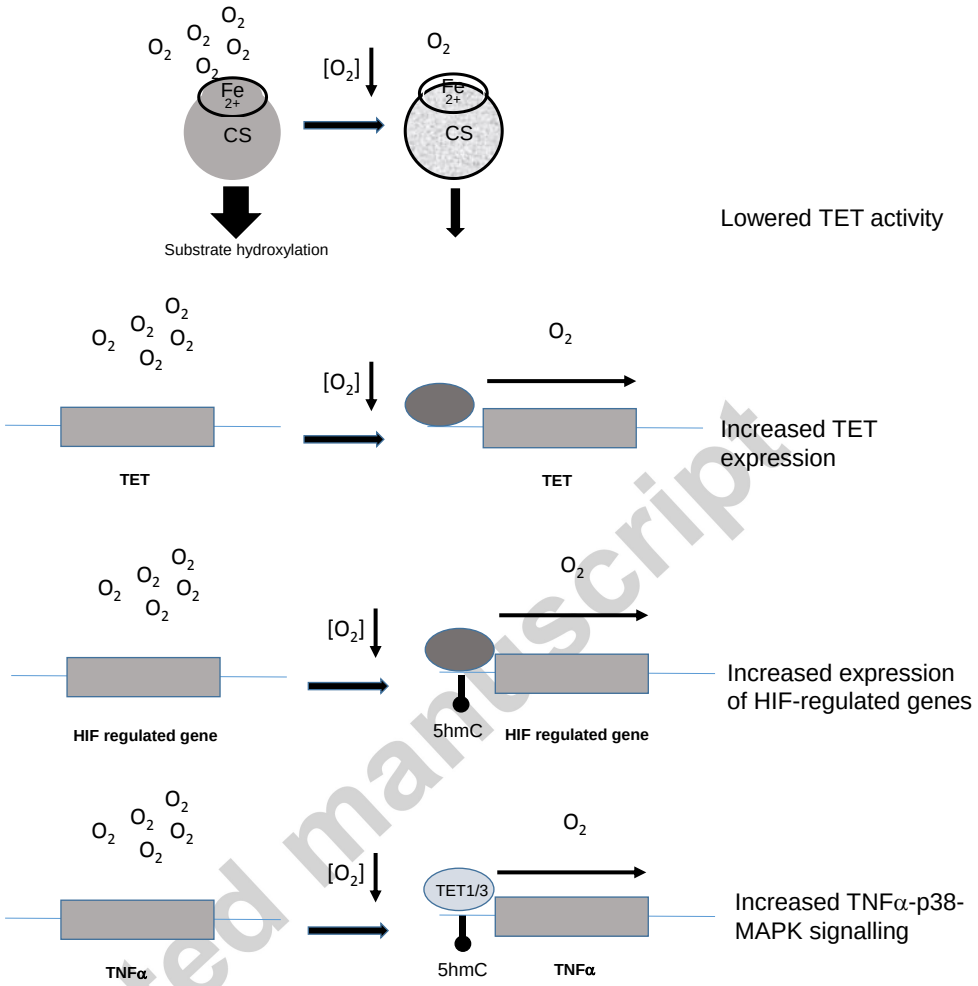
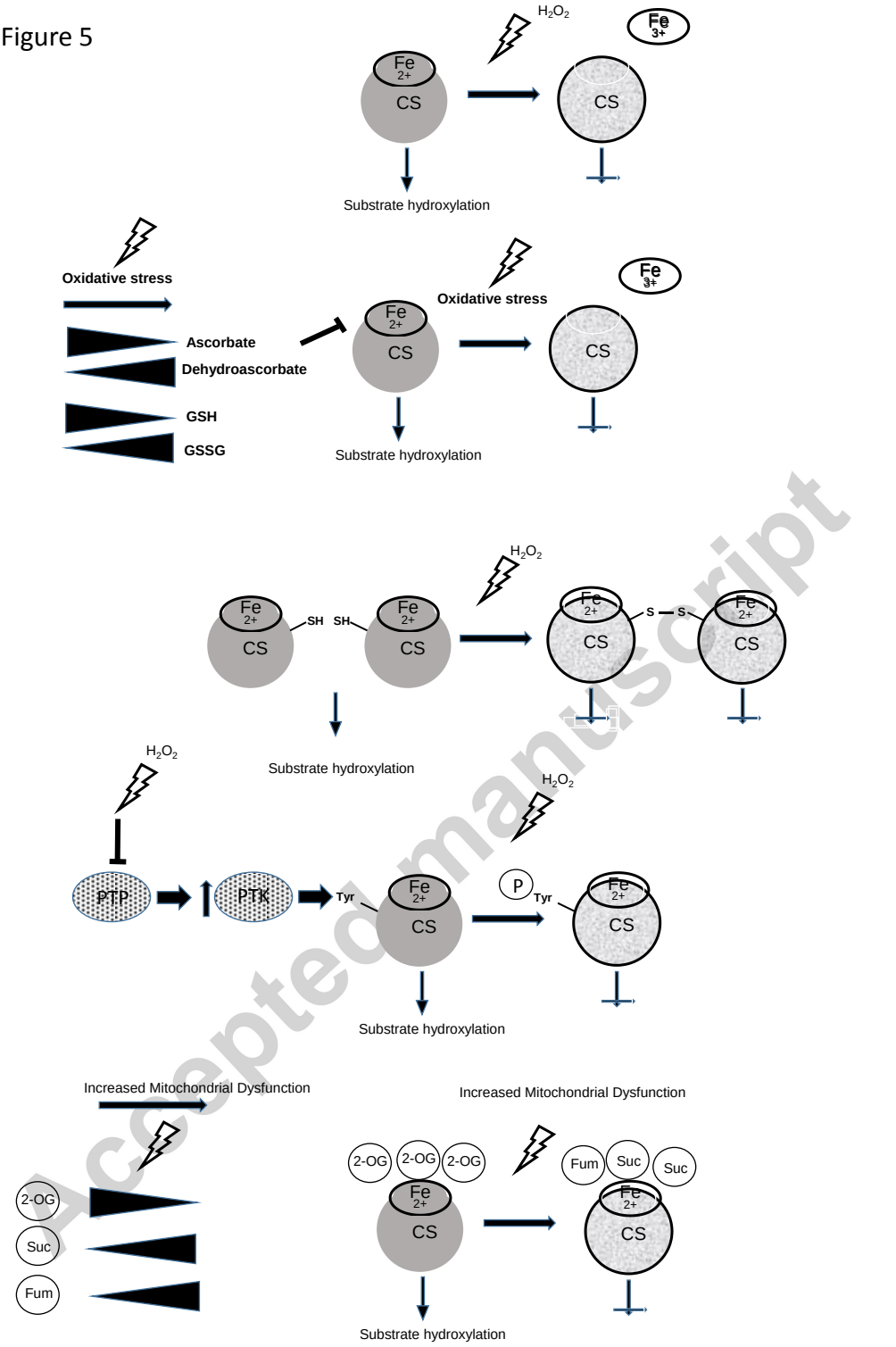
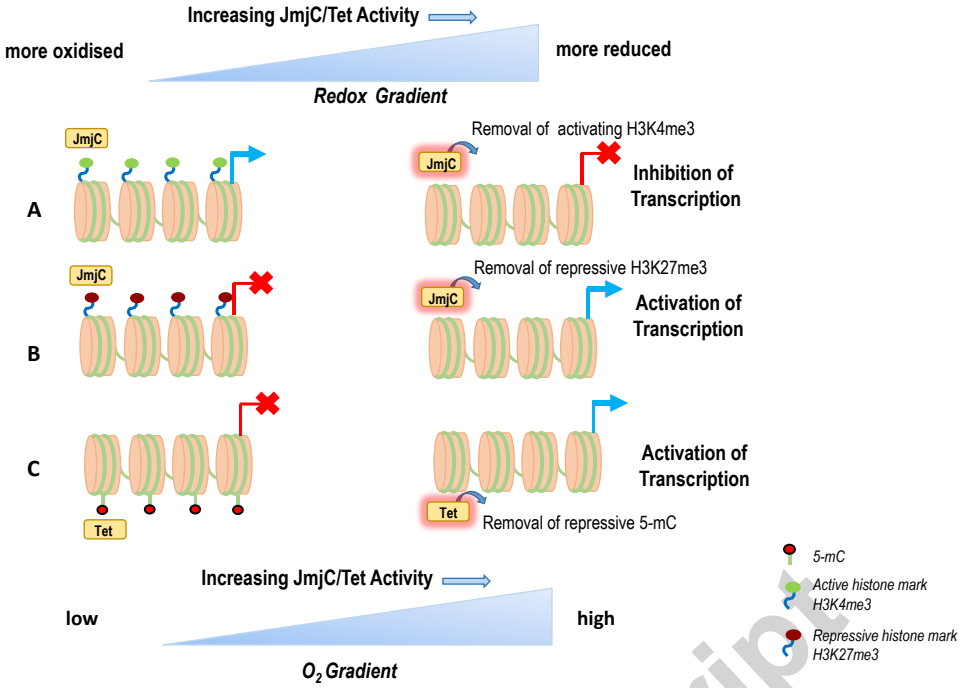


Figure 5





Graphical abstract

Highlights

- Gene transcription is regulated in part by epigenetic erasers which act to remove methyl groups from DNA and histones, termed TET and JmjC proteins, respectively
- Both TETs and JmjCs belong to a superfamily of proteins which are 2-oxoglutarate- and Fe(II)-dependent dioxygenases
- Consequently the *activities* of these enzymes are regulated in part by cellular redox and O_2 availability
- TETs and JmjCs can therefore facilitate the transduction of dynamic changes in cellular redox and O_2 concentration with changes in gene expression